LamdaGen's LSPR-Based Plasmonic Biosensors *vs* Gold Colloids and other optical technologies: The Sensing Range & Quality Factor

The aim of this brief is to provide a description of the optical properties of LamdaGen's gold plasmonic sensor surfaces and compare them with the optical properties of gold (Au) colloids. As we will show, the plasmonic films are superior to gold colloidal solutions for biosensing. In fact, the optical properties of the plasmonic films match those of single Au nano-rods, one of the most sensitive techniques for biosensing as interrogated a single particle at a time. While biosensing at the single molecule level poses enormous challenges commercially, the patented manufacturing process of the company's continuous nano-structured thin-films is highly reproducible, scalable and economic. Thus, the plasmonic films bring the benefits of the nanoscopic world into the commercial realm.

Localized Surface Plasmon Resonance (LSPR)-based sensing is a technique that uses nanometer-size metallic structures to detect biomolecular interactions with simplicity and exquisite precision. Seemingly, LSPR-based biosensing devices can be easily fabricated. In fact, a collection of gold (Au) colloids of diameter below ~100 nm or simply a single gold colloid are examples of such devices. The first generation of diagnostic products, such as lateral flow strips, relied on the strong absorption of these gold colloids. These tests use a capture antibody immobilized on a precise location of a strip of chromatographic paper and detector gold colloids conjugated to a secondary antibody dried on a membrane upstream from the location of the capture antibody strip. Addition of the sample fluidizes the gold colloid conjugates and drives them across the antibody test strip where they can become immobilized via an antibody-antigen sandwich bridge if the sample contains the antigen. If sufficient detector gold colloids become immobilized at the test strip, they become visible to the naked eye. Since the mid 1980's, rapid lateral flow tests following this principle have been developed for a plethora of conditions from pregnancy testing, to assessment of bacterial infection, to detecting the presence of allergens. These lateral flow tests are rapid and cost effective, but otherwise qualitative (or semi-quantitative) in nature and limited to the >10s of ng/mL level of detection (LOD).

There is however a second sensing mechanism using Au colloids that is vastly superior to absorption: it relies on the spectral shift of light absorbed or scattered by metallic nanostructures, often referred to as the plasmon peak. Yet, for commercial diagnostics, the inhomogeneity of colloidal Au solutions produces a large broadening of the plasmon peak that ultimately reduces sensitivity. This has prevented significant acceptance of LSPR technology as a viable commercial solution for the next generation of rapid point of care (POC) diagnostics.

LamdaGen's continuous nanostructured Au thin-film biosensors are a formidable alternative that alleviate the shortcomings of gold colloids. The plasmonic films are made of a thin gold film with surface patterns defined at the nanoscale level via a proprietary manufacturing technique.[1]
In bio-sensing, two of the most important properties of plasmonic devices are

- the **sensing range**, which defines the distance from the surface above which the device is insensitive to perturbation
- the **quality factor Q**, which measures the narrowness of the resonance

It turns out that LamdaGen's plasmonic films are characterized by much narrower and intense plasmon resonances compared to solutions of Au nanoparticles and single Au nanospheres. These phenomena results in a far higher Q factor and shorter sensing field that negates spurious artifacts often referred to as bulk effect. Bulk effect is something that has hampered the use of SPR and other surface sensing techniques in diagnostics.

In this note, we will compare the sensing range of the LamdaGen platform to other optical detection platforms. We will discuss the implication of the very short sensing range of LSPR for the development of a simple and rapid bioassay at the POC.

Sensing Range and Bulk Effect

LamdaGen's continuous nano-structured films can be pictured as an array of small antenna located at the surface of the metal. The antenna field is not confined at the interface, but can extend up to a certain distance into the solution, as illustrated in Fig.1. This distance is technically called the evanescent field, and we will refer to it as the sensing range.

Every inhomogeneity, perturbation or fluctuation that occurs within this sensing range can perturb the reception and generate a parasite signal. For optical biosensors, these include changes in refractive index of the sample due to temperature fluctuations or gradients (~ 0.1-1°C for SPR), presence of biological aggregates from complex matrixes, presence of enzymes or signal amplification moieties. The parasite (unwanted) signals are often termed as "bulk effect".

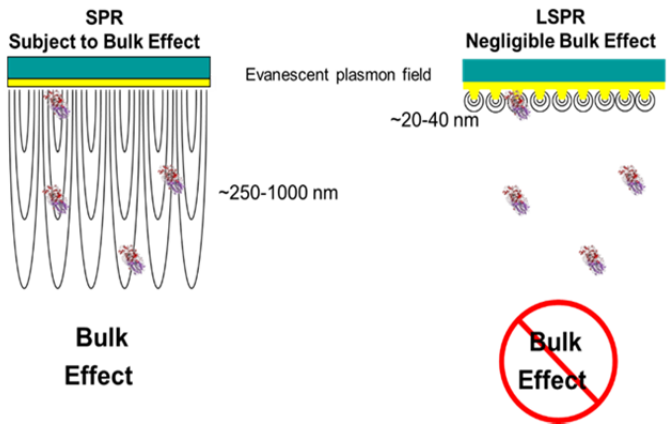


Figure 1 Illustration of the evanescent field concept for SPR and LSPR. Biomolecules within the range of the evanescent field contribute to the signal, even though they are not bound to the functionalized surface. For instance, in SPR the 4 molecules contribute to the signal even though only one is bound to the surface. In LSPR only the bound molecule contributes to the signal. In addition, fluctuations in index of refraction within the evanescent field due to temperature fluctuation, diffusion of aggregates or others also contribute to the signal. These events are not related to any specific binding events and thus constitute a parasite signal, often called "bulk effect".

An ideal biosensor reacts only to an analyte binding to the functionalized surface and nothing else. Thus an ideal biosensor should respond to events occurring only 5-10 nm away from the surface (the size of a biomolecule) and must be blind to anything further out in the solution.

The sensing range can be experimentally measured by growing multilayers of electrostatically bound polymers extending from the sensor surface (alternating positively and negatively charged polymers) as explained in Fig. 4 and in Ref [2]. Each layer has a well-defined thickness and sits at a well-defined distance from the interface. At a certain point, the additional layers fall outside the surface's sensing range and the readout signal levels off.

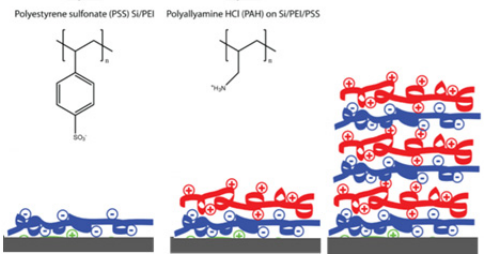
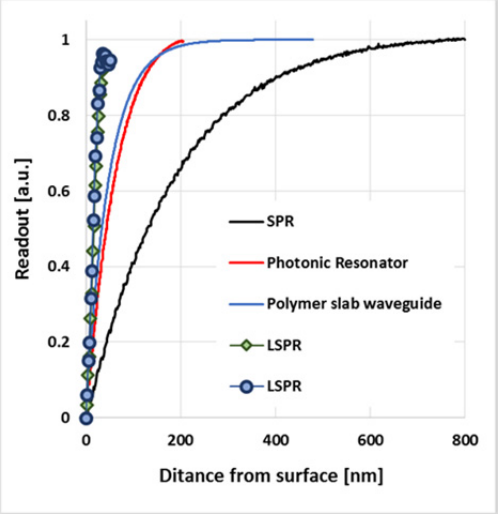


Figure 2 Adapted from Ref [2]. Schematics for the multilayer build-up used to probe the evanescent field of a biosensor. After the addition of each layer, the differential response of the biosensor is measured. Since each layer has a well-defined thickness, it is possible to correlate response vs distance from the interface.

Figure 3 reports the experimental sensing range for four biosensing platforms: LSPR, SPR[2] and a silicon photonic resonator[3] and a polymer slab waveguide.[4] It is clear that the sensing range of a *localized* surface plasmon biosensor is much shorter than the sensing range of SPR and of a photonic resonator or a polymer waveguide. Here, we

quantify the sensing range as the distance at which the response reaches 90% of saturation. Based on this simple criterion, we experimentally deduce a sensing range for LSPR of ~32 nm, while it is over 110 nm for all other technologies



Sensing modality	Sensing Range @ 66% of saturation [nm]	Sensing Range @ 90% of saturation [nm]	Ranges of Values found in the litterature
LSPR	19	32	20-40
SPR	200	400	250-1000
Polymer slab waveguide	52	110	
Photonic Resonator	62	120	80-150

Figure 3 Measured evanescent fields for LSPR, SPR, a photonic resonator and a polymer slab waveguide - Adapted from Ref. [2, 3, 4]. The values of the evanescent field depend slightly on the experimental conditions and each single biosensor, and the criteria used to compute the sensing range. Thus we also report values found in the literature. Often, the sensing range is determined through an exponential fit through the curves of in Fig. 3 and the sensing range is equated to the exponential constant which represents the distance from the surface where the readout is at 66% of saturation. By this definition, 33% of the sensing volume still contributes to the parasite signal even though it is far away from the surface. We elect to use a more stringent criterion for the sensing range, as the distance where less than 10% of the signal may originate away from the surface.

In summary, biosensors are sensitive to what occurs in the vicinity of their surface, but the meaning of "vicinity" varies greatly between platforms. Ideally, biosensors should be sensitive only to events ~ 5-10 nm away from their surface to differentiate between biomolecules bound to the surface through a capture ligand and to biomolecules free-floating in solution. As shown above, LamdaGen's plasmonic films exhibit the shortest sensing range. A very short sensing range has important beneficial consequences for biosensing. In a typical blood/serum diagnostic sample, the biomarker titer is usually too low for robust detection using a simple label-free assay format, i.e. when the binding of the biomarker itself is directly detected. Signal amplification is required. It turns out that a platform with a short sensing range is amenable to signal enhancement for low abundance targets in a single step, no wash assay that are impossible to do with platforms having larger evanescent fields.

Q Factors and Dephasing Plasmon Times

First, we compare in Fig. 4 the plasmon extinction spectra of LamdaGen's plasmonic films to the extinction spectra of commercial 20nm and 40nm Au colloids. The spectra have not been corrected. It is clear that while colloids and films have resonance extinction peaks at the same energy (or wavelength), the width of the plasmonic films' resonance is far narrower.

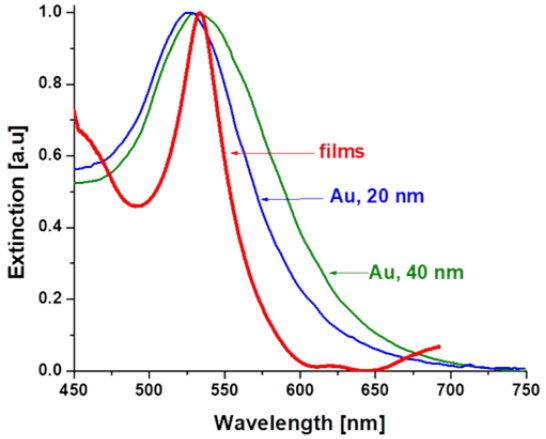


Figure 4 Comparison between the extinction spectra of 20 nm and 40 nm Au colloidal solutions from a commercial source to the extinction of plasmonic films from LamdaGen. Structurally, the plasmonic thin-films integrate billions of Au nanostructures in an area of ~ 10 mm^2.

	E_R [eV]	Γ [meV]	Quality Factor Q	Dephasing time [fs]
Au, 20 nm	2.36	398	5.93	3.31
Au, 40 nm	2.33	436	5.34	3.02
LSPR films	2.32	153	15.20	8.61
LSPR films	2.27	155	14.67	8.50
LSPR films	2.34	169	14.07	7.79
LSPR films	2.36	179	13.22	7.36
LSPR films	2.36	180	13.17	7.32
LSPR films	2.27	163	13.93	8.08

Table 2 Experimental Q factors and dephasing times of colloidal gold solutions and LamdaGen's plasmonic nanostructured thin-films.

Spectra similar to those shown in Fig. 4 allow the computation of two important experimental parameters: a) the plasmon linewidth Γ, identified here as the full-width at half maximum of the resonance, and b) the resonance energy E_R, identified as the location of the maximum of the resonance. From the knowledge of the resonance linewidth Γ and energy E_R, we can define the quality factor Q of the resonance as

$$Q = \frac{E_R}{\Gamma} \qquad (1)$$

From the linewidth Γ, it is possible to extract the dephasing time T_2 of a plasmon excitation, i.e. the time it takes for damping a plasmon:

$$T_2 = \frac{2\hbar}{\Gamma} \qquad (2)$$

Spectra similar to the ones shown in Fig.1 have been measured for multiple biochips as part of the company's QC process, each biochip consisting of eight plasmonic spots. The plasmon spectra of the eight spots of the same biochip essentially overlay, and therefore we ascribe a single Q factor for the entire 8-spot biochip. Typical values for the quality factor and the dephasing time of a few biochips are reported in Table 1. The quality factor of LamdaGen films are in the 13-15 range, i.e. 2.5 to 3 times higher than those of colloidal solutions. As a consequence, the plasmon dephasing time is 3 times larger in films than in solutions.

It is instructive to compare the Q values and dephasing times with values measured for *single* gold nanospheres and nanorods in darkfield microscopy,[5] perhaps the ideal model system for plasmonics. In a 2002 pioneering work, Carsten Sonnichsen and coworkers measured the plasmon resonances of single Au nanospheres of various diameters (20 nm to 150 nm) as well as those of gold nanorods with various

aspect ratios (short axis 15-25 nm, long axis up to 100 nm) using darkfield microscopy. While plasmon resonances of Au nanospheres are degenerate due to symmetry, the nanorods exhibit different resonances along their short and long axes. The resonances along the short-axis resemble those of spherical particles, but the ones along the long-axis present a broader interest since they have a large intensity (oscillator strength) and a lower resonance energy.

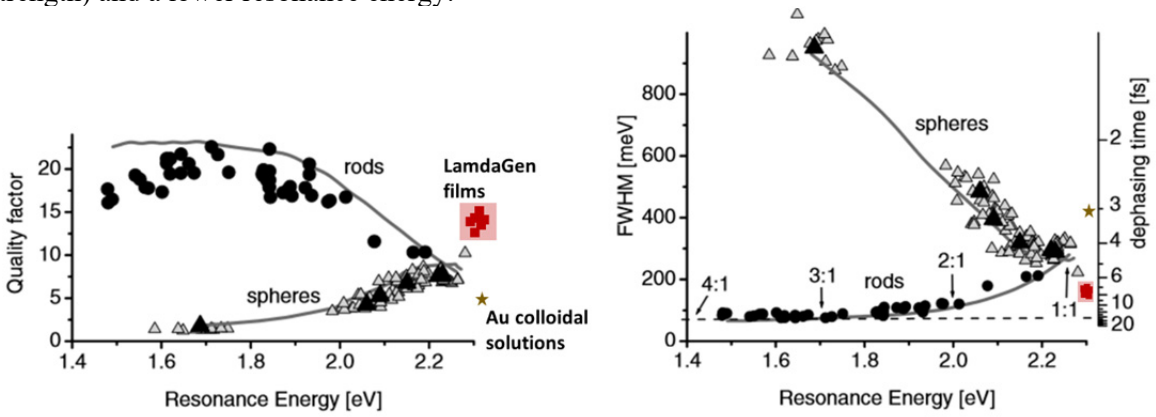


Figure 5 Adapted from Ref [5]. *Left:* The quality factor Q as a function of plasmon resonance energy for Au spheres and nanorods. The Q factor of LamdaGen films is represented by the red squares and the ruby area, while commercial Au nanoparticle solutions are represented by the yellow star. Notice how the Q factors of LamdaGen films stand out in the plane: they have high Q factor, and their resonance frequency is in the visible range in contrast to Au nanorods, which have high Q factors but are in the near infra-red range, which is incompatible with the imaging sensitivities of low-cost digital cameras.
Right: Plots of the resonance linewidth and dephasing time. Again, LamdaGen plasmonic films have a long dephasing time in the visible spectrum in sharp contrast with commercial Au solutions or single nanorods.

In Figure 5, we adapt Fig. 4 of Ref [5] that summarizes the Q factors of single nanoparticles and nanorods to include the Q factors of LamdaGen's plasmonic films. Fig. 5 showcases beautifully that the continuous plasmonic films are in a league of their own. In fact, the Q factors and dephasing times of LamdaGen films are close to the Q factors and dephasing times of highly anisotropic single nanorods. There are however two differences to bear in mind relative to commercial applications.

1. To fully harness the high Q factor of the nanorods, the excitation light must be polarized along the long-axis of the nanorods. Practically, this poses a number of challenges since this can only be achieved by aligning all the nanorods in a sample and using a light polarized along the long-axis of the aligned nanorods, or as an alternative, by using randomly-oriented nanorod samples and detecting at the single molecule level. Neither of these options is scalable nor commercially viable.
2. Nanorods have a resonance frequency of 1.5 – 2.0 eV which is in the red to near infrared range (800 nm – 620 nm), i.e. outside the sensitivity range of low-cost digital cameras.

In summary, LamdaGen's plasmonic Au films have the highest Q factors in the visible range of any plasmonic platform developed in academia or commercially. The higher Q factor of films *vs* colloidal solutions translates into increased sensitivity for biosensing as the change in plasmon resonance frequency is proportional to the Q factor. The Q factor of LamdaGen films is far higher than the one of colloidal Au solutions, and on par with that of *single* Au nanorods with a large aspect ratio. In contrast to the synthesis of Au nanorods, where non-uniform size and shape distributions are unavoidable, LamdaGen's proprietary process yields homogenous films of macroscopic dimensions (0.1 – 3 mm) that are highly reproducible, scalable and economical.

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