Austin Journal of Biosensors & Bioelectronics

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Editorial

Nanohole Biosensor-Origin and Application as Multiplex Biosensing Platform

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Received: June 22, 2015; **Accepted:** June 23, 2015; **Published:** June 25, 2015

Editorial

One of the primary challenges faced by the present day diagnostic industry is to develop novel strategies for analysing multiple samples in parallel without compromising the target specificity and Limit of Detection (LOD). This requirement has led to the emergence of assay platforms that can efficiently analyse multiple samples or to put it into context, a sample containing multiple bio-markers with a single test. These new generations of assay methods are commonly known as multiplex bioassays. Clinical diagnostic industries embracing this multiplex platform are capable of offering simultaneous analysis of up to 100 biomarkers within a single sample. The target analytes are more often customizable and include (but not limited to) metabolic, immune/auto-immune, isotyping, cell/tissue-typing, cytokines, and genotyping.

The modes of detection for multiplexed bioassays mainly are absorbance, fluorescence, Raman scattering, and Surface Plasmon Resonance (SPR). However, one bottleneck for various multiplex techniques occurs when multiple targets are present in an analyte with a highly disproportionate ratio. The presence of trace amounts of key analytes may remain undetected or more commonly get masked by some abundant co-analytes. Hence, very often vital information may be potentially lost as a key component remains undiagnosed. This limits the very basic requirement of multiplex bio-assays, which is to detect all the target analytes efficiently from a mixture.

To address this limitation, scientists have come up with an approach, which can significantly improve the detection efficiency for trace analytes by enhancing the optical signals (transmission) by introducing an assay platform involving a specifically patterned noble metal nano surface. This platform, which is an extension of traditional SPR methodologies, is known as Enhanced Optical Transmission (EOT) and the patterns formed on the noble metal surfaces are arrays of nanoholes with defined diameter and periodicity. This generation of assay platform or sensors is known as 'Nanohole Biosensors' [1].

EOT-Breaking the Dogma

In aperture theory an old school of thought was that the amount of transmitted light through an aperture in a metal sheet (necessarily opaque) should be inversely proportional to the hole-area. This notion was built upon some interesting optical experiments performed by Bethe et al during 1930s [2]. In this work, he showed that, if the hole diameter is smaller than wavelength of transmitted light, then transmittance, $T \alpha (d/\lambda)^4$ (d=hole diameter and λ= wavelength). This classical aperture theory was based on a single hole in an infinitely thin slab and was further extended to real metals and hole arrays.

However, it took the physicists nearly six decades to discover something very different. In 1998, Ebbesen's research group performed a pioneering experiment on the transmission of light through nanohole arrays fabricated on Gold and Silver thin films [3]. They observed a significant deviation from the classical aperture theory as the amount of transmitted light was observed to be significantly larger than that predicted for particular wavelengths. They also observed that the noble metal thin film had become more transparent than predicted. This is indicative of more light transmission than the amount impacted on the hole. This highly unpredicted observation was termed as Extraordinary Optical Transmission (EOT). However, the effect gradually wore off when noble metals were replaced by other metals. The maximum transmission peaks were found to be a function of the nanohole periodicity. From the outset, EOT looked like a potential game-changer in optical instrumentation, especially for high precision sensing devices (Figure 1).

Understanding EOT- Many Key Players

The next big step was to understand the phenomena properly to make maximum use of EOT in device fabrication. Before divulging

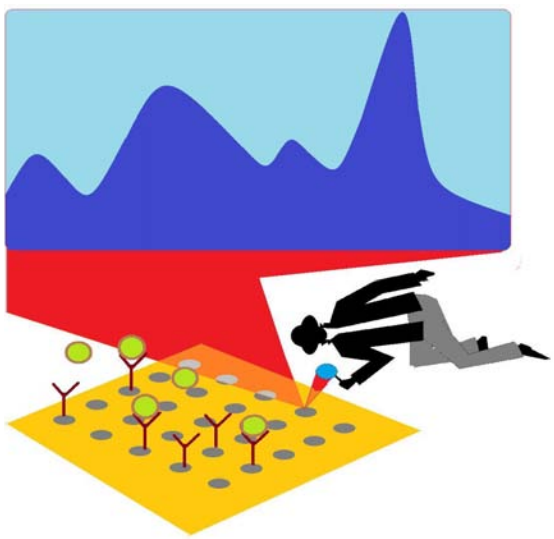

Figure 1: Nanohole biosensor is a high throughput optical platform for improving state of the art bio-assay sensitivity.

Citation: Roy S and O'Mahony J. Nanohole Biosensor-Origin and Application as Multiplex Biosensing Platform. Austin J Biosens & Bioelectron. 2015;1(3): 1012.

into the theories behind EOT through nanohole arrays, it is essential to understand the Surface Plasmon Polariton (SPP) or in short, Surface Plasmon (SP) itself. It will also be important to understand how differently a single nanohole interacts with the SP compared to the nanohole array.

SPPs are described as collective charge oscillations produced by the resonant interaction between free electrons and light at the interface of dielectric materials and metallic surface. It was mathematically derived by solving Maxwell's equations under the condition where the in-plane vector, *k* that is continuous at the interface between semi-infinite metal surface having complex di-electric function (di-electric constant ε_m) and the di-electric medium (di-electric constant ε_d) displays the interaction between refraction and absorption. This is given in equation 1.

$$k = \frac{\omega}{c}\left(\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}\right)^{1/2} = k_{SP}$$

It is quite evident here that the dielectric functions, ε_m and ε_d must be of opposite sign if '*k*' needs to be a real component assuming the strong conduction behaviour of ε_m. In this situation, the magnitude of k_{SP} will always be larger than the photon ($k_L = \omega/c$) - making the coupling between light and SP not feasible. Hence, the primary requirement would be to increase the value of wave vector of photons to be equal to the value of k_{SP}.

Two theories were proposed to aid the coupling [4]. The first one is based on Attenuated Total Reflection (ATR) theory based on the Kretschmann–Raether geometry. Under this optical condition, the dielectric function of the dielectric medium increases significantly over that of the air, increasing the photon momentum leading to (angularly modulated) coupling between the SP and photon. The second theory is based on the coupling of grating momentum. Grating structures are created by the presence of periodic structures (i.e. periodic arrays of nanohole in this case). This grating momentum is inversely proportional to the lattice parameter of the system, which corresponds to the measure of periodicity of the nanohole arrays. Hence, for an optimized small value of array periodicity, photonic and plasmonic vectors can undergo efficient coupling leading to EOT. In reality, it is probably the interplay of both the mechanisms that results in EOT, where the optical configuration determines which mechanism dominates. Here also lies the difference between the optical behavior of nanohole arrays compared to that of the single holes. Single holes or rather isolated holes are devoid of such grating momentum coupling due to the unavailability of any periodicity. Hence, only the first mechanism (ATR) is operative here. However, a detailed description of the electromagnetic coupling phenomena around a single elliptical aperture in an optically thick metal was provided by Zakharian [5]. This theory was further modified by several groups by extending the phenomena to LSP (localized surface plasmon)-photon coupling, LSP-SPP coupling and its polarization dependent nature are strongly supported by electro-dynamic simulation approaches [6].

EOT Mediated Improvement of Bio-Diagnostics

The two most important aspects of clinical diagnostics are the visualization of target physiological phenomena and their sensitive quantification. Fortunately enough, EOT provides both. The most sensitive mode of light microscopy (UV/vis to IR range) to date is considered to be an optimum combination between fluorescence microscopy and dark field microscopy providing higher range of fluorescence signal to noise ratio as well as lower background compared to conventional fluorescence microscopy. Techniques like confocal microscopy, fluorescence lifetime imaging microscopy used to be a benchmark for this type of detection approach. The inclusion of EOT provides nano-antenna structures with grating capabilities to further improve the resolution paving the way towards a super-resolution microscopy platform. Concomitant with improved detection, the assay performance has improved further with 10 to 15 times enhancement of fluorescence signal due to EOT [7]. An additional three-fold increase of assay sensitivity was achieved by optimizing the angle of incident light on the sample. Further improvement was achieved when fabrication of single-hole nano-arrays have been graduated to double-hole nano-arrays as shown by Lesuffleur *et al.* [8].

Fabrication of Nanohole Array- Lithography Approaches

The recent advances in nanofabrication techniques have revolutionized our ability to design and create a patterned nanosurface with desired optical properties. Depending on the detection requirements, both the

'close-ended' (for flow-over operation mode) as well as 'through' (for flow-through operation mode) nanoholes are being fabricated for the last two decades using a variety of lithographic techniques. Some of these lithographic approaches are worth mentioning to give an idea of the state-of-the-art nanofabrication [9].

Colloidal (micro/nano) lithography

This simple and relatively economic technique requires colloidal nano or micro particles (polystyrene, silica etc) as mask. At first a monolayer of the beads are achieved (by spin coating). Then it undergoes controlled metal coating using thermal evaporation. Finally, beads are peeled off using adhesive tapes or controlled sonication to expose metallic hole-arrays as a footprint of the 'up-rooted' beads. However, this method very often suffers from the lack of a sufficiently large coverage of hexagonally close packed monolayer distribution.

Focussed ion beam (FIB)

This high resolution, mask less and direct writing technique uses a low energy ion beam (ion sources as Ga, Au-Si-Be) as the milling tool. The beam removes material from the metal surface by collision thereby creating holes. The hole diameter, depth and shape is controlled by tuning the beam intensity.

Electron beam lithography (EBL)

This writing technique is associated with the SEM (scanning electron microscopy) and utilizes a focussed electron beam for the purpose of milling. EBL can either be a direct writing method or it may follow lift-off procedures. This technique is usually hailed as more versatile and better suited for high-density nanohole array fabrication.

Nanoimprint lithography (NIL)

This multi-step writing technique involves fabrication based on imprinting a film of polymer with a mould. Having the capability of fabricating both the ‘dead-ended’ and ‘open-ended’ nanoholes, this method uses a negative mould with nanostructures as the stamp on a thermo-plastic or UV-curable surface. This technique is highly sensitive to temperature and pressure at the various stages. The pre-imprinting, imprinting, post-imprinting mould detachment stages require individual critical temperatures.

More recent methods include high through-put template stripping and Lift-off Free Evaporation (LIFE) techniques which are gradually being introduced to the nano-fabrication industry.

Conclusion

Nanohole biosensors are potential game changers in the multiplexed biosensing industry. They are capable of developing very high resolution microscopy techniques as well as bio-assay platforms with multi-fold improved assay sensitivity. Conventional as well as novel fabrication approaches are constantly widening its horizon with improved optics and smallest possible foot-print with maximum sensitivity.

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Austin J Biosens & Bioelectron - Volume 1 Issue 3 - 2015
ISSN : 2473-0629 | www.austinpublishinggroup.com

Citation: Roy S and O’Mahony J. Nanohole Biosensor-Origin and Application as Multiplex Biosensing Platform. Austin J Biosens & Bioelectron. 2015;1(3): 1012.