The Pennsylvania State University
The Graduate School

Department of Chemistry

DESIGN AND DEVELOPMENT OF NANOSTRUCTURED SURFACES FOR ENHANCED OPTICAL SENSING

A Dissertation in
Chemistry

by
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ABSTRACT

At smaller size regimes, materials’ physicochemical properties change with respect to bulk analogs. In the case of metal nanoparticles like gold or silver, specific wavelengths of light can induce a coherent oscillation of their conduction electrons, generating an optical field confined to the nanoparticle surface. This phenomenon is termed surface plasmon, and has been used as an enhancing mechanism in optical sensing, allowing the detection of foreign materials at small concentrations. The goal of this dissertation is to develop nanostructured materials relying on surface plasmons that can be combined with different optical sensing platforms in order to enhance current detection limits.

Initially, we focus on the development of surfactant free, stimuli responsive nanoparticle thin films, which undergo an active release when exposed to a stimulus such as a change in pH. These nanoparticle thin films provide faster analyte particle transport and direct electronic coupling with the analyte molecule, all without attenuating the evanescent wave from the optical transducer to the particle. These stimuli responsive nanostructured substrates are tested within a surface enhanced Raman platform for the detection of biomolecular probes at sub-nanomolar concentrations and µL sample
sizes. Furthermore, the developed nanosubstrates can be patterned, providing a versatile nanoparticle thin film for multiplexing analysis, offering a substantial advantage over conventional surface based nanoparticle detection methods.

Our results encouraged further optimization of light-matter interactions in optical detection platforms. It is for that reason that this dissertation evolves towards confined optical systems. Particularly, whispering gallery microcavities confine electromagnetic waves – at high volumes – at the boundary of a dielectric resonator. In this dissertation, we examined the sensitivity of whispering gallery modes combining optical microcavities with plasmonic nanoparticles in analogy to a “nanoantenna”. First, our hybrid methodology is tested by analyzing the resonant wavelength displacement of a whispering gallery mode cavity upon perturbation with a gold nanoparticle layer containing a model protein. Next, we developed a real-time optical sensing platform relying on whispering gallery microcavities and surface plasmons, and then tested it for the detection of a model protein at fM concentration (less than 1000 protein molecules).

Finally, this plasmonic-photonic coupling process involving whispering gallery modes is studied via a self-referenced methodology relying on the mode splitting of a whispering gallery resonance. Specifically, we studied the mode splitting evolution of a resonant whispering gallery microcavity as a function of gold nanoparticle adherence with varying diameters. Mode splitting increases as the localized surface plasmon wavelength of the nanoparticle approaches the spectral line of the whispering
gallery mode. Plasmonic-photonic coupling observed in this study provides a novel alternative to achieve single particle detection using mode splitting, as well as understanding optimization of particle size for plasmonic-photonic coupling.

The study described herein opens a new way to optimize current optical sensing technology, enabling not only the detection of an analyte, but also the execution of fundamental studies of analyte interactions at ultralow concentrations.
# TABLE OF CONTENTS

LIST OF FIGURES ............................................................................................................. viii

LIST OF TABLES ................................................................................................................ xv

ACKNOWLEDGMENTS ...................................................................................................... xvi

Chapter 1 Introduction ........................................................................................................ 1
  1.1 Light-matter Interactions ......................................................................................... 1
  1.2 Light-matter Interactions at the Nanoscale ............................................................ 6
  1.3 Light-matter Interactions from a Sensing Viewpoint ............................................. 8
  1.4 Scope of this Dissertation ....................................................................................... 12

Chapter 2 Material Synthesis of Quasiperiodic Plasmonic Nanoparticle Arrays .......... 17
  2.1 Plasmonic Nanoparticles ....................................................................................... 19
  2.2 Plasmonic Nanoparticle Synthesis ........................................................................ 22
  2.3 Stimuli-responsive Substrates ............................................................................... 26
  2.4 Protein Biomarker Detection ............................................................................... 34
  2.5 Detection of Viral Molecular Probes ..................................................................... 39
  2.6 Summary ............................................................................................................... 45

Chapter 3 Optical Resonances and Confined Systems: An Introduction to Whispering
  Gallery Systems ............................................................................................................. 47
  3.1 General Description of Resonator Systems ............................................................ 47
  3.2 Optical Microcavities and Whispering Gallery Resonances .................................. 51
  3.3 Hybrid Photonic-Plasmonic Microcavity Whispering Gallery Resonators ........... 59
  3.4 Protein-Particle detection with microcavity resonators ......................................... 62
  3.5 Microcavity-Particle coupling ............................................................................. 72
  3.6 Summary ............................................................................................................... 78

Chapter 4 Ultrasensitive Protein Detection Studies in Real-Time ....................... 80
  4.1 Molecular Detection in Real-Time ......................................................................... 80
  4.2 Real-Time Protein Detection Platforms Utilizing WG Modes ............................ 83
  4.3 Results ............................................................................................................... 87
  4.4 Optical Trapping .................................................................................................. 93
  4.5 Summary ............................................................................................................. 105
Chapter 5 Single Plasmonic Nanoparticle Detection in a Whispering Gallery Microcavity ................................................................. 107
  5.1 Whispering Gallery Mode Resonances – An introduction to Mode Splitting ........................................................................... 107
  5.2 Single Plasmonic Particle Detection – Experimental Methodology .......... 112
  5.3 Single Plasmonic Particle Detection – Results and Discussion ............... 119
  5.4 Summary ................................................................................................. 126

Chapter 6 Conclusion and Future Work .............................................................. 128
  6.1 Conclusion ............................................................................................... 128
  6.2 Future Work ............................................................................................ 133

Bibliography .................................................................................................... 136
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The Lycurgus cup exhibiting (A) reflected and (B) transmitted light. The cup shows Lycurgus being enmeshed by Ambrosia. ©Department of Prehistory and Europe, The British Museum. Height 16.5 cm, diameter: 13.2 cm. Reprinted with permission from the trustees of the British Museum.</td>
</tr>
<tr>
<td>2.1.1</td>
<td>Schematic diagrams illustrating (A) a localized surface plasmon and (B) a propagating surface plasmon across a metal-dielectric interface, also termed as surface plasmon polariton.</td>
</tr>
<tr>
<td>2.3.1</td>
<td>(A) Schematics describing the steps of nanoparticle substrate preparation via a galvanic electroless process of silver, Ag, on a 4.5 nm thick germanium, Ge, evaporated on a quartz substrate. Top surface AFM images of (B) quartz, (C) germanium on quartz, and (D) Silver/germanium on quartz are shown. From: Langmuir 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.</td>
</tr>
<tr>
<td>2.3.2</td>
<td>(A) Extinction and (B) EDS spectrum of silver/germanium substrate. The EDS spectrum is collected after ODT wash, which removes the silver nanoparticles from the surface. The instrument used for EDS acquisition consisted of a Philip XL-30 scanning electron microscope (SEM) equipped with an Ametex attachment; the SEM was operated at an accelerating voltage of 5 kV to minimize sample damage. From: Langmuir 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.</td>
</tr>
<tr>
<td>2.3.3</td>
<td>(A) AFM image of the germanium surface (0.5 x 0.5 micron) which clearly shows the nanoposts structure that forms after 30s immersion in a 2 mM silver nitrate solution. AFM image is recorded after removing the silver nanoparticles from the surface by ODT surfactant. (B) Etch depth of the germanium surface at various times of the nanoparticle synthesis. The red curve shows a trendline, approximated to $t^{1/2}$, for the etching rate of germanium. From: Langmuir 2012, 28 (14),</td>
</tr>
</tbody>
</table>
Figure 2.3.4  (A) Extinction spectra of silver nanoparticles on germanium film (black) in air and silver nanoparticles released to the acetic acid solution (pH = 6.0) after 2 seconds (dots) and 800 seconds (dashes) of immersion. Inset spectra show the extinction of released silver nanoparticles in time series (2, 100, 200, 400, 600, and 800 seconds after the immersion). The progress of the acquired spectra is shown in time from low to high extinction. (B) Time series spectra showing the absorbance of silver nanoparticles released into a tris(hydroxymethyl)aminomethane (Tris) buffer solution (pH = 8.0). Spectra are shown for 2, 4, 10, 20, 50, 100, 150, 200, 300, 400, 500, and 600 seconds (time progressing from low to high extinction) after immersion into the buffer solution. Illustration in the inset describes the optical configuration employed for monitoring the released nanoparticles (plane view). From: Langmuir 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012. .......................................................................................................................... 31

Figure 2.4.1  Crystal structure of the AAL protein, extracted from http://www.rcsb.org/pdb (PDB code: 1IUC). Crystal data regarding the structural information of this protein can be found in the work developed my Mike et al.75 .......................................................... 32

Figure 2.4.2  SERS spectra recorded at 633 nm of (black) the recombinant versions of AAL, (blue) recombinant version of AAL with L-fucose, and (red) L-fucose. Reproduced with permission of the IEEE copyright 2011. ......................................................................................................................... 39

Figure 2.5.1  (A) Schematic diagram describing steps of microarray preparation, Molecular probe conjugation, hybridization of the target sequence, and detection. (B) Arrays of micro-patterned nanoparticle layer. (C) 1 µl droplet on one of the microarray spots is shown, (D) TEM image of the silver (Ag) nanoparticles after analyte induced aggregation and drying. From: Langmuir 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012. ........................................................................................................................................... 42

Figure 2.5.2  Molecular probes attached to the responsive active release nanoparticle substrate based on the schematics described in Figure 2.5.1. (A) RSV and HBV gene detection is tested on a
microarray format using a Renishaw Raman microscope. Raman signals show clearly distinct spectra of Cy5, especially around 1200 cm\(^{-1}\) and 1600 cm\(^{-1}\) which are the fingerprint peaks for Cy5 dye on the molecular probe. (B) The sensitivity of the RSV-Cy5 shows a detection limit of 100-1000 molecule. (C) Molecular probe specificity of RSV-MP tested using two target sequences (RSV and HBV) which are designed based on Table-2. RSV target results in loss of the SERS signal but HBV target shows Cy5 signal. (D) Schematic diagram illustrating the molecular probe cross-reactivity assay. Adaptation from: *Langmuir* 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.

**Figure 3.2.1** Schematic depiction describing the constructive (A) and destructive (B) interference path on the cross-sectional plane of a WG optical cavity.

**Figure 3.2.2** Schematic diagram describing the planar view of an optical WG microresonator. A tunable laser is employed to send electromagnetic waves to a photodetector, and an optical fiber is used to guide that light into its final destination. The thinned (tapered) region of the fiber generates an optical field that evanescently propagates into the medium. WG modes are excited by confining the evanescent waves of the fiber inside the resonator structure, which is accomplished when the microcavity is brought into mechanical contact with the fiber. The excitation of WG modes is evidenced as dips in the transmission signal captured by the photodetector.

**Figure 3.4.1** (A) Schematic diagram describing the fabrication of the gold (Au) nanoparticle (NP) template containing BSA. (B) Electron microscopy images of bare AAO and AAO with Au nanoparticles using an FEI Philips XL-20 microscope. Adapted from: *Applied Physics Letters* 2011, 99 (7), 073701. Reproduced with permission of the American Institute of Physics copyright 2011.

**Figure 3.4.2** Schematic diagram of the set-up employed to evanescently couple WG modes to the Gold nanoparticle (NP) layer. Top: WG mode transmission spectrum for the microsphere in air. Bottom: WG mode spectrum for microsphere in contact with nanoparticles which induce WG mode wavelength shift (continuous line). Reproduced with permission of the American Institute of Physics copyright 2011.
Figure 3.4.3  An example of a WG mode spectrum in air (dotted line) and after evanescent coupling to a BSA-nanoparticle layer (continuous line). Reproduced with permission of the American Institute of Physics copyright 2011. ................................................................. 67

Figure 3.4.4  WG mode measurements at ~633 nm probing wavelength. The inset shows the BSA adsorption at high solution concentrations compared to the dilute concentration in a log-linear graph. Reproduced with permission of the American Institute of Physics copyright 2011. ................................................................. 69

Figure 3.4.5  WG mode control measurements at two different wavelengths (633 nm and 1064 nm) for gold nanoparticles immobilized on an AAO membrane and gold nanoparticles immobilized on a silicon dioxide slide. ................................................................. 70

Figure 3.5.1  A cross-sectional representation of the system used in these calculations. ................................................................. 74

Figure 3.5.2  (A) Spectral shifts of the first-radial-order WG mode of a 5µm-diameter silicon dioxide (SiO₂) microsphere in air caused by the adsorption of a 55 nm-diameter gold nanoparticle (shown in the inset). (B) Intensity enhancement as a function of wavelength on the gold nanoparticle surface in the presence (red dots) and absence (blue line) of the sphere under the illumination by a linearly polarized plane wave. (C) Q-factors of the resonances corresponding to the WG mode in the microsphere and hybrid photonic-plasmonic modes in the coupled microsphere-nanoparticle structure. (D-E) Two-dimensional spatial electric field intensity distributions at the wavelengths of a hybrid resonance, λ₀ = 630.84, (D) and the corresponding WG-mode resonance, λ=630.819 nm, (E) – microsphere surface is shown as a dashed line. Reproduced with permission of the American Institute of Physics copyright 2011. .......................................................................................................................... 77

Figure 4.3.1  (A) Schematic diagram of the wetted gold nanoparticle layer coupled to a WG mode resonant microcavity. (B) Protein detection scheme by monitoring the wavelength shift of a resonant microsphere cavity. (C) A photograph of the WG mode setup mounted on a high-precision stage located on an inverted microscope. (D) SEM image of gold nanoparticles immobilized onto an AAO nanoporous membrane. Reproduced with permission of John Wiley and Sons 2012. ................................................................. 88
Figure 4.3.2 An example of a BSA adsorption curve, upon BSA addition, the resonance spectra shift drastically and BSA adsorption is observed in real time as an increase in resonance wavelength. Reproduced with permission of John Wiley and Sons 2012. ................................................................. 89

Figure 4.3.3 Sphere-normalized WG mode shift measured at 633 nm nominal probing wavelength of the gold nanoparticle layer. Reproduced with permission of John Wiley and Sons 2012. ........................................ 90

Figure 4.3.4 Rate plot of the sphere-normalized fractional wavelength displacement signal corresponding to the different adsorption kinetics of BSA measured in real time for citrate and amino-modified gold nanoparticle layer. ........................................ 92

Figure 4.4.1 (A) Schematics and two-dimensional spatial field maps of (A) the gold nanoparticle cluster (trimer) excited with external illumination, and (B) a hybrid microsphere-nanoparticle (gold) cluster sensor under the plane wave illumination at 634.9 nm. Reproduced with permission of John Wiley and Sons 2012. ........................................................................ 94

Figure 4.4.2 Fractional wavelength shifts of the hybridized WG-plasmonic nanoparticle mode of the sensor caused by the adsorption of BSA molecules one-by-one. The inset shows the positions of the adsorbed molecules relative to the hot spots formed in the nanoparticle cluster. Reproduced with permission of John Wiley and Sons 2012. ........................................................................ 96

Figure 4.4.3 (A) Field intensity enhancement in a hybrid structure composed of a microsphere coupled to a gold nanoparticle dimer as a function of the nanoparticle diameters and wavelength under plane wave illumination (as shown in the inset). The intensity is evaluated in the nanoparticle dimer gap (detector position shown as a red dot). (B) Intensity enhancement spectra of gold nanoparticle dimers of varying nanoparticle diameters and their spectral overlap with the WG mode of the isolated sphere (shown as dash line). (C) Intensity enhancement in the hybrid sphere-cluster configuration as a function of the cluster size. The inset shows the positions of nanoparticle (gray circles) added to the cluster randomly in the order indicated by their numbers and the position where the field enhancement was evaluated (red dot). (D) Intensity enhancement spectra of gold nanoparticle clusters of varying size (labels indicate the number of nanoparticles in the clus-
ter; nanoparticle diameter is 40 nm) and their spectral overlap with the WG mode of the isolated sphere (shown as a dashed line). Reproduced with permission of John Wiley and Sons 2012.

**Figure 4.4.4** The field intensity map for random gold nanoparticle is shown. This map is calculated using the generalized Mie-theory. Representative MC simulations results for N=50 and N=500 are shown in (B) and (C) respectively. (D) BSA coverage, at the hotspot locations, shows clearly a different trend for the light-field on (blue) and the light-field off (red) MC simulations. Reproduced with permission of John Wiley and Sons 2012.

**Figure 4.4.5** BSA coverage, at the hotspot locations, as a function of gold nanoparticle diameter. Reproduced with permission of John Wiley and Sons 2012.

**Figure 5.1.1** (A) Cross-sectional plane view of a doubly degenerated WG resonator exhibiting two propagating modes, clockwise (CW) and counter-clockwise (CCW), as well as the single Lorentzian resonance signal at resonant wavelength ($\lambda_0$) and with linewidth ($\gamma_0$) from the transmission spectrum collected at the photodetector site. (B) Cross-sectional plane view of a doubly degenerated WG resonator affected by a scattering center (i.e. inhomogeneity, geometrical imperfection, particle, etc.), which lifts the original degeneracy and splits the resonance signal into a doublet. The resonance wavelengths and linewidths of the modes in the doublet are denoted as $\lambda_{1,2}$ and $\gamma_{1,2}$ respectively.

**Figure 5.2.1** Schematic representation of the instrumental components utilized in these experiments. In essence, a tapered fiber is used to guide the light from a tunable laser into an optical resonator; a photodetector collects transmitted light at the other end of the fiber, and the transmitted signal is monitored. The inset shows a typical doublet in the transmission spectrum. The resonance wavelengths and the linewidths of the modes in the doublet are denoted as $\lambda_{1,2}$ and $\gamma_{1,2}$ respectively.

**Figure 5.2.2** Schematic diagram (not scaled) describing a detailed protocol for the fabrication of high-Q ($Q > 10^7$) silicon dioxide microtoroids.
Figure 5.2.3  Optical image capturing the instrumentation utilized in this study. The inset shows WG modes excited at 660 nm on a microtoroid resonator containing gold nanoparticles. ................. 116

Figure 5.2.4  Two-dimensional matrix showing the transmitted field collected at the photodetector site. The horizontal axis denotes the frame number corresponding to the spectral acquisition range, whereas the vertical axis indicates the frame number related to the acquisition time. (B) Example illustrating how the developed data processing program works; in this case, the program was run over the whole spectral range (horizontal frames, 1 to 10000) and for each vertical frame (1 to 2500); the three images show extractions from the initial, middle and final frames (left to right) displaying the fitting (green line) according to Equation 5.2.1 (green line). ................. 116

Figure 5.2.5  (A) Conceptual illustration of the plasmonic nanoparticle detection experiments. The difference in mode splitting of a microtoroid WG mode cavity is analyzed over time as gold nanoparticles are deposited on the surface of the microcavity. The particle size is varied to understand the effect of the particle diameter in the induced polarizability at the microresonator surface. (B) UV-Vis absorbance spectra of the gold nanoparticles utilized in these experiments. The inset plots the measured LSP as a function of particle diameter. (C) The change in the wavelength separation (i.e., amount of splitting) (left) and the linewidths (right) of the resonance split modes as a function of time. Single particle binding is reflected as discrete sudden jumps in the amount of splitting and linewidth difference. .................................................. 117

Figure 5.3.1  (A) Polarizability volume distribution for 30 nm gold particles adsorbed on the surface of a microtoroid WG mode resonator illuminated at 660- and 1450- nm respectively. (B) Average polarizability volume, (C) average detected particle diameter, and D average particle count for gold particles (20-, 30-, 50-, and 100- nm) as a function of the particle size for the sensing experiments done at 660- and 1450- nm. ........ 125
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table 2.4.1</th>
<th>SERS peak assignments for L-fucose and rAAL</th>
<th>38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.5.1</td>
<td>Designed RSV and HBV molecular probes</td>
<td>41</td>
</tr>
<tr>
<td>Table 2.5.2</td>
<td>SERS peak assignment for Cy5 molecular probe</td>
<td>45</td>
</tr>
</tbody>
</table>
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This dissertation is dedicated to my family, my greatest blessing.
A Aurora, mi madre;
a José, mi hermano;
y a la memoria de Ramón, mi padre.
Los amo.
Chapter 1

Introduction

1.1 Light-matter Interactions

The interaction between light and matter has been considered as one of the most fundamental aspects of science. Understanding what light is, what matter is, and the interaction between the two has been one of the most critical steps towards the boost of technology, as we know it. Nowadays, we do not only possess a fundamental understanding of light, we are able to create it, confine it, transport it, utilize it to probe materials, gaining information in return, and even employ light to transmit information. Of course, this would not have been possible without understanding the interaction of light with matter. The previously mentioned accomplishments are the results of hundreds of years of investigation, and the progress generated has catalyzed a technological revolution, contributing to the development of computers, communications, and electronics.
Light-matter behavior engages a prominent portion of our current technologically centered world. However, before diving into more details of the interaction between light and synthetic materials, it is worth mentioning the importance of light to life. In fact, it is fair to admit that light is responsible for what we – nowadays – refer to as life. Light is a small portion of the electromagnetic spectrum, which can also be conceptualized as energy that propagates through free space or a material medium. Long before the appearance of complex organisms in nature, microscopic organisms were able to harvest energy from light in order to sustain themselves, creating, in consequence, a suitable scenario for the rising of higher hierarchies of life.

One of the most notable examples of energy harvesting is the photosynthesis, which consists of a solar energy storing process. Basically, solar energy is absorbed by a naturally occurring biomolecule that serves as a molecular antenna, chlorophyll; the absorbed energy is then converted to chemical energy – at a reaction center – in the form of energy-storing molecules, which are utilized to drive cellular processes.\(^1\) Another interesting example is the iridescent blue color exhibited by the blue *Morpho rhetenor* butterflies; which is not due to pigmentation. The wings of the blue Morpho are made chitin, a transparent polymer, and the intense blue color is due to photonic structures formed by discrete multilayers of chitin and air.\(^2\) Little is known about the evolution of this biological nanostructures; however, this evolutionary design serves as an inspiration to scientists and engineers for the development of materials with enhanced physicochemical properties.
Another light harvesting system, which occurs in biological organisms, is the eye. In fact, eyes should be considered as one of the most outstanding complex optical sensors that nature has ever created. Analogously, sensors usually have a detection mechanism that basically captures the information of interest, and a transducing mechanism that translates the detected information into something useful. In this case, eyes are commonly defined as organs of vision that absorb light (detection event) and transduce it into electrochemical impulses with the ultimate goal of providing information about the surroundings, yielding a unique perception of reality.

The existence and evolution of eyes along with the intrinsic curiosity of the human-kind has enabled the exploration of the different interactions between light and matter. From a condensed point of view, when light interacts with matter, light can excite the atoms and molecules of that material, which is known as absorption; it can bounce, this process is termed reflection; it can change its direction, i.e. light traveling from the sky into a lake, which receives the name of refraction, and it can deflect into multiple directions, this is recognized as scattering.

As one delves into more profound details of the behavior of light, one can certainly establish that the interaction of light with an object can be ranked into parametric or elastic and non-parametric or inelastic processes. The first one group all those interactions that do not inflict a change in the state of the physical entities involved in the composition of a material (also referred as quantum state); hence, there is no change
in incoming or outgoing energy, nor momentum. Non-parametric processes, on the other hand, refer to those ones that alter the quantum state of the system. If light is considered as a transverse propagating wave of photons in the visible realm of the electromagnetic spectrum, and photons are conceptualized as elementary particles defining the quantum form of electromagnetic radiation; the gain of a fundamental conceptualization of the surroundings becomes more accessible by understanding how these visible electromagnetic waves interacts with materials. When light is absorbed the object takes up the electromagnetic energy. Specifically, the photons excite the valence electrons of atoms, and depending on the excitation transition there are different relaxation mechanisms (i.e. luminescent processes like fluorescence or phosphorescence, or thermal processes). Reflection is another interesting outcome observed when a light wave interacts with matter and diverges its trajectory without affecting the quantum state of the system. Additionally, electromagnetic waves can bend their trajectory as transitioning into a different medium (i.e. air-water) while conserving the frequency, this process is known as refraction and it is responsible for the visual distortion when looking at underwater objects from an air interface. Finally, electromagnetic waves can deflect into multiple directions after colliding with an object, if there is an energy or momentum transfer between the photons of the irradiating wave and the physical object, the process is referred as inelastic scattering, if the energy and momentum of the system are conserved then it is termed elastic scattering.
The behavior of light within a material is given by the way in which light travels in the material, which is accounted for by a parameter called index of refraction (n), or $n = c/\nu$, where $c$ is the speed of light in vacuum and $\nu$ is the phase velocity of light in the material. The index of refraction not only indicates how fast light travels within a material, but also how light is bent or refracted in a given medium. Earlier in this section, light is defined as a transverse propagating wave; specifically, light has a magnetic component (TM) and an electric component (TE), which propagate transversally to the direction of the propagating wave and normal to each other. From a material properties perspective, the index of refraction also accounts for the interaction of a given material with both the TM and TE fields, which is described by the relative electric permittivity ($\varepsilon_r$) of the material, and relative magnetic permeability ($\mu_r$), according to the equation $n = \sqrt{\mu_r \varepsilon_r}$. However, it should be taken into account that in the vast majority of naturally occurring materials, the atoms are mainly affected by the TE field of light, not the TM field. Therefore, it is normal to observe that the magnetic permeability very rarely deviates from one, whereas the electric permittivity ranges between positive and negative values, depending on the material. In addition, the values of the permittivity and the permeability are never simultaneously negative in nature, that is all naturally-occurring materials have a positive index of refraction, approximately to $n = \sqrt{\varepsilon_r}$. This is the reason why a light wave incident on the material from an air interface at an off-normal angle is refracted to the opposite side of the interface normal. Understanding the different relationships that light and matter may exhibit, has enabled the foundation of methodology to harvest, and manipulate light for a vast majority of purposes expanding from clinical to energetic applications.
1.2 Light-matter Interactions at the Nanoscale

Humankind has brilliantly succeeded in the ways light is manipulated, gaining precise control on light-matter interactions. This progress in combination with advances in material synthesis has enabled a field known as photonics.

Nanoscale objects often display physicochemical properties that are not usually observed in bulk analogs. From a chemical perspective, the gain in surface area of nanoscale objects has enabled the development of nanosubstrates with enhanced catalytic behavior as compared to bulk. A notable example can be found in the interaction between light and metal nanoparticles. While a large piece of gold or silver exhibits a yellowish (gold) and gray (silver) color respectively, gold and nanoparticles may exhibit almost any color in the visible spectrum. The reason for this phenomenon is the propagation of localized surface plasmons (LSP). Metals have delocalized electrons, which give them the ability to conduct electricity, and that particular shine when exposed to light. Gold and silver nanoparticles also have conducting electrons, in fact, at such size regimes, the conductive electrons behave as free charge across the surface of the particle. Thereby, electromagnetic waves of a given frequency can drive the excitation of LSPs by inducing a coherent oscillation of those delocalized electrons or free charges in the particle, arising a dipole-like oscillation at a given incident frequency. A notable aspect of LSPs is that the resonant frequency can be easily tuned by changing the shape, size, and composition of the nanoscale object (i.e. nanoparticles).
cle, nanowire, nanoribbon, etc.); this interesting quality allows one to carefully design and develop a nanomaterial based on the probing energy regime required for a given study.

In essence, the fundamental behavior of LSPs and a nanomaterial can be conceptualized as an enhancing interaction occurring between the incident light and the nano-object, which in consequence creates an electromagnetic field confined to the vicinity of the nanomaterial. In other words, these metal nanostructures are able to accumulate, concentrate and confine energy in very small volumes, acting as a “nanoantenna”, and enabling a vast number of applications that have unified distant areas of knowledge such as biology, chemistry and physics resulting in the development and growth of hybrid disciplines and fields of learning. The discovery of LSPs has given birth to plasmonics, which is one of the most active subareas in the photonics research field, expanding from molecular recognition and sensing to photochemistry and energy-related research.

Society’s ever-growing energetic demands require the development of novel ways to generate energy in a sustainable manner. Plasmonic nanomaterials show an excellent potential to address limitations in the development of novel materials for renewable energy applications. The optical field confined at the surface of a plasmonic nanoparticle enhances light absorption and emission, as well as heat exchange. In terms of optical or plasmonic enhancement, metal particles with tunable surface LSPs have been utilized to harvest light in broadband dye-synthesized solar cells, as well as in
energy storage chips. Additionally, it should be noted that the light accumulated at the nanoparticle site also heats up its surroundings, acting like a source of available energy in the form of heat that can be used to catalyze chemical transformations in confined environments.

### 1.3 Light-matter Interactions from a Sensing Viewpoint

The ability to generate and manipulate light in combination with the understanding of light-matter behavior have facilitated the growth of different methodologies that rely on the fundamental interactions (absorption, scattering, refraction, radiative decays among others) between light and a given material.

One of the earliest examples of molecular recognition can be attributed to the study of the transmission decay of an incoming light beam after interacting with a material. This absorption process has allowed one to design platforms to trap and detect analytes at bulk concentration levels. Further research has boosted a myriad of techniques that allow not only the ultra-sensitive detection of molecules but also their trapping and manipulation.

Another great example of light-matter interactions that can be applied to molecular sensing is fluorescence. In this particular case, the material absorbs light at a given frequency, exciting the electrons of the material; fluorescence is observed when the
system relaxes via a radiative mechanism, which consists of the return of those excited electrons to the ground state releasing a photon in the process. The understanding of this process has enabled a robust methodology that is currently used in numerous bioassay protocols for the labeling, subsequent identification and quantitative analysis of specific analytes. 12-13

The Raman effect is another notable example, which arises when light waves are scattered from a molecule; when this occur, most photons are elastically scattered; however, a small portion of the light is scattered in an inelastic manner due to changes in vibrational, rotational or electronic energy of the molecule. Those inelastically scattered photons constitute the Raman effect, which is the pillar of Raman spectroscopy (RS); and have proven their value in the chemical characterization of molecular backbones. 14-15 Nonetheless, one of the most notable limitations of RS resides in its low scattering efficiency only 1 in 10 million photons scatters inelastically,16 which severely limits the application of this process for molecular sensing at ultralow concentrations. Interestingly, the inclusion of metal nanoparticles annihilates RS limitations by inducing a physical enhancement due to the propagation of surface plasmons. The combination of plasmonic particles and RS gives rise to surface enhanced Raman spectroscopy (SERS), one of the most powerful spectroscopic techniques capable of detecting analytes at low concentration levels.17-26

Spectroscopic techniques often require the collection of light at a detector site over a given period of time. Typically, as the concentration of the analyte is lower, a longer
collection time is needed in order to get an accurate spectrum. Surface plasmons can circumvent this limitation; furthermore, the localized fields generated via LSPs can be virtually combined with a vast number of optical detection mechanism in order to enhance their intrinsic sensitivity.

The development of surface plasmon resonance (SPR) sensor technologies represents another extraordinary contribution. It should be noted that SPR relies on the utilization of surface plasmon polaritons (SPPs), which are electromagnetic excitations propagating at the interface between a dielectric and a conductor, and evanescently confined in the perpendicular direction to the propagation; as opposed to LSPs, which do not propagate from the particle surface. In SPR a light source is focused through a prism, reflecting on the back side of a sensor surface (usually a thin metal film) and eventually travels into a detector; at a certain incidence angle (resonance angle), light is absorbed by the electrons in the metal film exciting SPPs as a consequence. SPPs are extremely sensitive to their surrounding environment and any changes in refractive index. The excitation of SPPs results into an optical loss in the reflection beam at the resonance angle and can be observed as a decay in reflection intensity signal. When a foreign analyte with a different refractive index is introduced into the system the there is a shift in the reflectivity curve, which is directly proportional to the amount of analyte at the sensor surface. The arrival of SPR sensors has enabled the detection of analytes in real time at extremely low concentration, avoiding the necessity of labels. SPR sensors have been widely investigated and continuous periodic nanostructures have been proposed as an alternative, increasing
sensitivity in SPR sensors through the propagation of localized surface plasmon resonances (LSPR), which provides a higher intensity in the localization of the optical field. As with every technique, SPR and LSPR are affected by the low resolution of the resonance signal and the susceptibility to thermal fluctuations. However, SPR and LSPR have proven to be successful methodologies to characterize reaction kinetics and affinity, as well as additional thin film properties, with a high degree of sensitivity and in a real-time manner.

All these examples describe sensing methodologies dependent on an exclusive interrogating interaction between light and an analyte, which is furtherly enhanced by the accumulation of a confined optical field at the surface of a metallic substrate. In the previously mentioned instances, light is used to confirm the presence of a given analyte. Recent progress in the optics research field has allowed the development of optical traps, capable of drawing the trajectory of highly polarizable particles and eventually capturing them in three dimensions.

One notable example is the development of optical tweezers by Ashkin and coworkers,\textsuperscript{30-33} which are employed to manipulate and execute fundamental studies of single particles and molecules. Analogously, photonic resonators can accumulate light at high field intensities; in such structures, light waves are internally reflected, and propagates through the resonator structure; in consequence, an evanescent field is generated at the boundary of the resonator material. The high optical field intensity
that occurs at the boundary of the resonator surface suits them as extraordinary candidates for optical traps, as well as exceptional transducers for molecular recognition in an ultrahigh sensitive manner.$^{34,45}$

### 1.4 Scope of this Dissertation

This dissertation describes novel methodologies that exploit the interaction of light with materials at extremely small scales and focuses on the design and development of nanostructured materials for enhanced optical sensors in order to achieve higher sensitivity. The utilization of light to rationally probe materials in a subwavelength size regime has been an ongoing research effort in different fields of knowledge such as chemistry, material science, and optics. The field of photonics encompasses these disciplines, and the contributions provided by precedent and current research have yielded novel optical mechanisms that allow the manipulation of light in unique ways; leading to the development of devices and platforms that efficiently utilizes the energy of light to investigate materials in the nanoscale realm. The knowledge gained in this hybrid photonic domain has been pivotal to achieve a more effective understanding of the world from a physical and chemical perspective, enabling the study of materials from bulk to individual components through precise light-matter interactions. Here, we present recent progress on the rational design, synthesis, and characterization of nanoplasmonic materials for ultrasensitive optical detection, and we discuss
fundamental progress in the molecular detection research field. Particularly, this research hypothesizes that the implementation of optical nanoparticle in current optical sensing methodologies is a crucial step towards the detection of single molecules.

Chapter 2 is focused on the development nanoparticle arrays for enhanced spectroscopic detection. Here, it is demonstrated that the reduction and oxidation behavior of metal ions can be exploited to synthesize metal nanoparticles (i.e. silver) on the surface of a semiconductor thin film in a spontaneous manner. In this case, the semiconductor substrate serves as an electron donor. The precise control of this process yields the development of a monodisperse metal particle layer that is nested on an electron donor surface; and by carefully controlling the chemical interactions between electron donor surface as well as surrounding medium, active nanoparticle release can be triggered. These quasiperiodic metal particles are probed with a plane wave of a given wavelength, exciting a coherent oscillation of their free oscillating conduction electrons, which is referred elsewhere as surface plasmons. The precise and meticulous control of the particle size accompanied of the probing wavelength selection enhances the naturally-occurring scattering of the nanoparticles creating high electromagnetic field intensities on the nanoparticle layer, which commonly receive the name of hotspots. These substrates are utilized to detect a protein biomarker for hepatocellular carcinoma and other liver diseases, as well as specific deoxyribonucleic acid (DNA) sequences through SERS in a sensitive manner.\textsuperscript{23-24}
Chapter 3 introduces the concept of optical resonances, and confined optical systems. This fragment of the dissertation describes the utilization of silicon dioxide microcavities to confine a traveling electromagnetic wave in the form of a whispering resonance. The trapping mechanism occurs via the internal reflection of the traveling electromagnetic wave, and the propagation of the resonance signal in combination with constructive interference generate high-field optical modes at the boundary of the photonic microcavity, which is termed as whispering gallery (WG) modes. Therefore, WG resonators derive their unprecedented sensitivity from their ability to confine light at high field intensities in the form of optical resonances, generating an evanescent field propagating in the normal direction to the resonant propagating wave. When a foreign material – with a different refractive index that the medium – binds the surface of the WG resonator, it interacts with the evanescent tail, changing the effective area of the resonator surface in return, which translates into a shift in the resonance signal; this resonance displacement is directly proportional to surface density (surface area) of the foreign material, and thereby the mass or amount of material bound to the surface of the resonator. Herein, the combination of such photonic platform with plasmonic particles able to sustain LSP resonances is hypothesized to provide a unique way to enhance the evanescent field of WG resonators without drastically impacting their photonic lifetime. The sensitivity of our established hybrid WG photonic-plasmonic platform is tested with the detection of a model protein at pM concentrations. Additionally, a theoretical framework explaining the hybrid photonic-plasmonic coupling of WG mode is described in an attempt to understand the
details of the coupling interaction between plasmonic particles (i.e. gold nanoparticles) and the evanescent WG mode of a resonance microcavity. Particularly, these calculations provide mechanistic information that explains the enhancing interaction between the plasmonic nanoparticle layer and the optical WG microcavity, which is supported by probing below (in energy terms) the LSP peak.

Chapter 4 introduces the concept of real-time sensing. In this chapter, the WG photonic-plasmonic platform developed in chapter 3 is implemented it into a real-time assay. The sensitivity of WG modes is enhanced as evidenced by the detection of a model protein at ultralow concentrations (~fM region) in 1.5 µL aliquots, which provides less than 1000 molecules in solution. Additionally, the previously established theoretical framework is combined with Monte Carlo simulations to understand the details of protein adsorption at ultra-low concentrations, and how the WG photonic-plasmonic coupling is affected by the size of the nanoparticle template.

Chapter 5 builds on previous results in order to achieve single nanoparticle detection. We designed an experimental protocol to confirm in a more direct manner the findings provided by the theoretical framework established in chapter 3. Specifically, the response of a WG mode cavity is studied after being perturbed with gold nanoparticles of different diameters. Instead of single WG resonance propagation, a mode splitting resonance, excited in a toroidal microcavity, is used due to its higher sensitivity and its heterodyne nature, which represents an unequivocal way to detect the presence of a single scatterer without need for the deconvolution of thermal fluctuations.
The mode splitting evolution of a resonant WG photonic microtoroidal resonator is monitored as a function of gold nanoparticle adsorption with varying diameters. As indicated in chapter 3, the perturbation of the mode splitting is increased as the LSP resonance wavelength of the nanoparticle approaches the WG mode spectral line. The results from this chapter provide a detailed knowledge of photonic-plasmonic coupling in WG systems, opening a new venue that significantly paves the way for the exploration of the WG mode perturbation by a single plasmonic scatterer using mode splitting. The findings of this research can be applied to other scientific disciplines besides molecular detection; for example, in the optoelectronics research field, this photonic-plasmonic approach can serve as an excellent tool for the development of heat management systems, which are briefly discussed in future work section of this dissertation.

Chapter 6 briefly compiles chapters 1-5 and provides an insight into future implications of the hybrid photonic-plasmonic system for enhanced optical sensing as well as other advanced optical applications. Additionally, this chapter also outlines potential future endeavors associated with the incursion of novel materials for photonic applications.
Chapter 2

Material Synthesis of Quasiperiodic Plasmonic Nanoparticle Arrays*


The twentieth century was characterized, in terms of research, by a scientific revolution, which is evidenced, in part, by the synthesis and development of nanomaterials. Although back in the ninth century (B.C), earlier civilizations used nanoparticles for creating shining effects in Mesopotamian pottery; at the same time, it is unclear the knowledge these civilizations had about the nanoscale world.

One of the most impressive examples of nanoparticle utilization in ancient history is the Lycurgus cup, which was made in the fourth century (A.D.). The Lycurgus cup, shown in Figure 2.1, consist of a cup made of a dichroic glass, exhibiting a greenish color when illuminated from the front, and a reddish color when illuminated from behind. Further examination revealed that the dichroic behavior of the glass is due to an extremely fine and precise distribution of gold and silver nanoparticles embedded
in the glass matrix. Initially, it was believed that a contamination was responsible for the peculiar color of the cup. Nonetheless, subsequent experimentation confirmed that the composition of colloidal gold and silver in the cup was 40 and 300 ppm respectively, suggesting that a contamination or the mere addition of metal traces is not enough to achieve such remarkable effect. Chemical analysis indicated that small amounts of gold and silver should have been chemically and thermally treated, using proper methodology, to produce colloidal metallic nanoparticles within the glass matrix in a homogeneous manner. Although the Lycurgus cup is the most notable example of dichroic glasses from ancient history, it should be mentioned that there are additional examples of dichroic glasses dating from the same historical period.

Figure 2.1 The Lycurgus cup exhibiting (A) reflected and (B) transmitted light. The cup shows Lycurgus being enmeshed by Ambrosia. ©Department of Prehistory and Europe, The British Museum. Height 16.5 cm, diameter: 13.2 cm. Reprinted with permission from the trustees of the British Museum.
The evidence of nanoparticle synthesis and manipulation from such a distant time period hints an intellectual perception, at least, of a material’s chemical behavior. Although the chemistry knowledge from ancient historical periods has not been entirely transferred from civilization to civilization, current characterization techniques allow one to pinpoint material properties and to extrapolate the anthropogenic knowledge of a given era. Additionally, with all these gaps in the transfer of information from time to time, humankind has been able to not only replicate ancient techniques but also develop new knowledge. The progress made in nanoscience and nanomaterial synthesis permits the careful control of the shape, dimension, and chemical behavior of nano-objects, as well as the nanomaterial synthesis of almost every element of the periodic table. Finally, current material synthetic tools have also allowed one to design the behavior of nanoscale objects in a given environment, which has initiated the field of “smart” materials; in other words, the development of materials that exhibit an autonomous and collective response when triggered by a specific stimulus.\textsuperscript{49-50} 

The present chapter will focus on the synthesis and development of quasiperiodic and monodisperse plasmonic nanoparticle thin films, as a class of smart material, which exhibit stimuli driven response in the form of autonomous particle release.

### 2.1 Plasmonic Nanoparticles

As described in the introduction, some metals exhibit a coherent oscillation of electrons when excited at specific frequencies of light; this effect is termed surface plasmon. One of the main characteristics required to support surface plasmons is that the
material possesses a complex relative permittivity consisting of a negative real, and a small positive imaginary, at the irradiation frequency. Considering that the relative permittivity is a measurement of the resistance that a material encounters when interacting with an electric field in a given medium; the meaning of a negative relative permittivity relates to the ability of a given material to exhibit no resistance to the electric field.

**Figure 2.1.1.** Schematic diagrams illustrating (A) a localized surface plasmon and (B) a propagating surface plasmon across a metal-dielectric interface, also termed as surface plasmon polariton.

Light is a form of electromagnetic radiation and can be defined as a transverse propagating wave of photons with two components TE and TM. The fundamental energy (E) of light is defined by its frequency or wavelength as follows \( E = h \cdot f \) where \( h \) is the Planck's constant, a proportionality constant that conveys the energy in one photon of electromagnetic radiation to the frequency \( (f) \) of that radiation. Light-wave oscillations can be described in terms of a sinusoidal function, where the frequency can be related to the wavelength \( (\lambda) \) as follows, \( f = c/\lambda \). Therefore, there is a specific energy associated with each frequency or wavelength of electromagnetic radiation.
When electromagnetic radiation, of a given frequency, interacts with a metallic nano-object smaller than the irradiation wavelength, and with a negative permittivity at that frequency of radiation, the TE field excites the surface (delocalized) electrons of the metal nanostructure, inducing a coherent oscillation of those electrons as a result. Thereby, an electric field dipole is created on the surface of the metal nanostructure. Surface plasmons can be localized on the surface of the nanostructure (i.e. nanoparticle), or propagate across a continuous interface between a conductor and a dielectric (polarizable insulator) material. The first phenomenon is defined in chapter 1 as LSPs and the latter is termed SPP. Figure 2.1.1 illustrates the difference between LSPs and SPPs.

The field of plasmonics studies light-matter interactions between light and nanostructures. Advances in this research area have enabled a wide array of applications in the field of molecular recognition, and enhanced spectroscopies. Metal nanoparticles have played a crucial role – as optical enhancers – in the plasmonics research field. Although, metal nanoparticles have stimulated a broad interest in molecular sensing, as they enable high sensitivity enhancements in spectroscopic detection techniques (i.e. Raman); for all the advantages brought by the arrival of plasmonic there is still room for much improvement in terms of materials fabrication and processing.
2.2 Plasmonic Nanoparticle Synthesis

The vast majority of techniques currently utilized for the synthesis of nanoparticles can be categorized into solution or surface initiated synthesis. In the case of metal nanoparticles, the synthesis currently involves the reduction of metal ions, followed by a controlled nucleation-driven growth; such that particle dimension and shape can be meticulously restrained. One of the simplest, and extensively accepted, synthetic methodologies for the synthesis of plasmonic colloids is the Lee-Meisel method.\textsuperscript{55} In fact, this approach has been subsequently modified and adapted to synthesize nanoparticles with specific characteristics, such as size, aggregation state, and chemical behavior.\textsuperscript{18, 56-59} The Lee-Meisel method can be utilized to prepare highly concentrated (0.1 mM) gold and silver nanoparticle solutions. In this method silver and gold precursors, typically silver nitrate or hydrogen tetrachloroaurate, are dissolved in an aqueous solution, the solution is heated to a certain temperature under stirring; then a reducing agent, i.e. sodium citrate, is added to the reacting mixture. When sodium citrate is used as the reducing agent, the carboxylic functionalities from the citrate molecule can serve as an electron donor, facilitating the reduction of gold and silver ions. The growing mechanism of metal particles originates from the extremely large surface tension of the as-reduced metal atoms in solution, forcing neighboring atoms to nucleate into bigger particles; once the reduction step has culminated, the nucleation step begins, this step is responsible for the particle growth. Then, the surfactant molecules in solution act as a capping layer by adhering to the surface of the metal nanoparticle. Sodium citrate is often employed in plasmonic nanoparticle synthesis.
because it is an excellent reducing agent, and surfactant, as the carboxylate anion (rich in electron density) and the delocalized electrons of the metallic surface of the particle can undergo metal-ligand bonding. The ratio of metal salt to reducing and capping agent is typically adjusted to regulate the particle size. This methodology has been intensively utilized to synthesize plasmonic nanoparticles due to its simplicity, and ability to modify for specific experiments.

The Lee-Meisel protocol has proven to be a convenient methodology to synthesize metallic nanoparticles of different sizes due to its versatility and simplicity. However, a standing limitation of solution-based metal nanoparticle synthesis, in terms of optical sensing, resides on the use of surfactants. In this case, surfactant molecules, often used as capping layers to avoid nanoparticle aggregation, act as blockers of the electronic coupling between the nanoparticle and the analyte molecule, attenuating the evanescent field from the optical transducer to the particle. In addition, surfactants also contribute to the creation of ambient background noise, lowering the sensitivity and the resolution of the detection technique. Although methods for removal or of the nanoparticle surface coating have recently been reported, additional treatments are required and in order to avoid re-aggregation of the clean nanoparticles surface. Surfactant exchange methods are avenues that can still be considered, but these methods are expensive, laborious and lower the particle yield. Furthermore, for the case of corrosive metals like silver, storage of the prepared metal films is an issue since surface contamination such as carbonates or sulfides can decrease the activity of the surfaces for analyte adsorption and optical probe responses.
An alternate route to the previously mentioned limitation is to synthesize metal nanoparticles on a surface. Different methodologies have been established to develop surfactant free metal nanoparticles on surfaces, an example is the electrochemical reduction of metal ions on an electrode surface.\textsuperscript{61} However, metal nanoparticles synthesized on surfaces also have drawbacks associated with analyte transport limitations from solution to the surface, which impacts the sensitivity and detection time.\textsuperscript{62}

Presently, there is an ongoing need for the development of synthetic tools to fabricate surfactant free and mobile nanoparticles for highly sensitive molecular detection applications. As an option to bypass current limitations, we explored the development of a smart metallic nanoparticle layer synthesized on the surface of a semiconductor thin film, which acts as a responsive substrate for the active release of nanoparticles into the analyte solution for rapid molecular detection experiments.

In this study, quartz substrates were coated with 4.5 nm of germanium via PVD utilizing a thermal resistive evaporator operated under ultra-high vacuum conditions, \(\sim 10^{-9}\) Torr before deposition (evacuation), and \(\sim 10^{-7}\) Torr during deposition.\textsuperscript{63} A shadow mask was employed during the deposition to create a high throughput array by patterning the germanium film. The germanium thickness was monitored by a parallel quartz crystal microbalance (QCM) located adjacent to the substrate holder. Subsequently, silver nanoparticles were reduced on the germanium film via a redox reaction dominated by a galvanic (spontaneous) process. The as-prepared germanium thin films were immersed in a 2 mM
silver nitrate solution for 30 seconds. The reaction was stopped by a deionized (ultrafiltered) water rinse, followed by drying under a stream of nitrogen. This galvanic displacement process has been previously demonstrated for silicon\textsuperscript{64} and germanium\textsuperscript{65-66} surfaces or wafers. However, the resultant nanoparticles are permanently immobilized at the substrate surface by strong metal/semiconductor adhesive forces, which hinders the active release. On the other hand, the thin film approach (i.e. a thin layer of germanium) provides an active release of nanoparticles triggered by a pH change.

The as formed nanoparticles can be rapidly released into the analyte solution after selectively dissolving the semiconductor layer in the presence of a weak alkaline solution. The advantages of nanoparticle release are: (i) it allows rapid sensing due to the mixing of nanoparticles with the analyte in solution, via evaporation-induced thermocapillary convection\textsuperscript{67} (ii) it circumvents transport limitations of the analyte to the nanoparticle surface, which typically limits the sensitivity to picomolar concentrations for practical time scales\textsuperscript{68}, and (iii) upon mixing, the released nanoparticles (hydrophobic in nature) spontaneously aggregate in a reagentless manner. It should be noticed that nanoparticle aggregation occurs on a slower time scale than the adsorption of the analyte; therefore, when the nanoparticles aggregate, the analyte has been already adsorbed on the surface of the nanoparticle junctions, which acts as nucleation sites and as ultrahigh field enhancement sites (hotspots) for plasmonic coupling\textsuperscript{69}. 
Based on this approach, we developed a protein biomarker sensor as well as a nucleic acid biosensor that involve intimate surface contact of the nanoparticle with a molecular probe.

### 2.3 Stimuli-responsive Substrates

Before describing the specific applications of the developed methodology; it is necessary to describe in more detail, the dynamics of the silver reduction process on a sacrificial germanium thin film. When the germanium surface is exposed to silver ions (Ag\(^+\)) in solution, the germanium serves as an electron donor, which reduces Ag\(^+\) ions in close proximity to the surface. During this Ag\(^+\) reduction process, germanium is consumed and released as soluble GeO\(_x\)H\(_y\) in the interfacial region of the nucleating/growing metal particle,\(^63-64,70\) but elemental germanium remains under the silver/germanium interface, enabling the Ge-to-Ag electron transport. The dynamics of this process leaves the originally smooth germanium thin film in the form of an ensemble of \(\sim 4.5\) nm high germanium protrusions or nanoposts, each capped with a silver nanoparticle of average diameter \(40 \pm 3\) nm. Figure 2.3.1 illustrates the details of this process (2.3.1A), including atomic force microscopy (AFM) scans of the quartz (2.3.1B), germanium (2.3.1C) and silver/germanium (2.3.1D). Analysis of Figure 2.3.1(D) gives a nanoparticle surface density of \(130 \pm 15\) particles per \(\mu m^2\) with a 2-D packing density, \(\phi\), of \(0.65 \pm 0.03\) (approximately equal to maximum random crystal
packing $\phi = 0.63$ on a surface). A nearest neighbor, $R_n$, analysis was executed according to Equation 2.3.1, and revealed a local order parameter smaller than 0.5,\textsuperscript{71} consistent with a random nucleation of nanoparticles at defect sites (e.g., grain boundaries, step edges) on the polycrystalline germanium surface at the initiation of the galvanic process.

$$R_n = \frac{D_{obs}}{1/2 \sqrt{\chi}}$$

Eq. 2.3.1

Where $D_{obs}$ represents the mean observed neighbor distance, $\theta$ defines the area under study, and $\chi$ accounts for the total number of points or specimens under study.

The reduction of Ag$^+$ ions on germanium thin films via galvanic displacement provides a uniform distribution of single nanoparticles on an ensemble of germanium nanoposts, with the advantage that the open structure enables rapid and reproducible particle release upon wetting with a weakly alkaline analyte buffer solution; this peculiarity provides a tremendous advantage when compared to typical thermal evaporation growth of silver nanoparticles on surfaces.\textsuperscript{61} The latter is a stochastic process that leaves randomly sized and distributed silver nanoclusters across the entire surface; additionally, our approach consists of a reagentless process, avoiding the need to use poisonous chemicals or electrochemical plating baths.

The unique structure of our nanostructured film is controlled by a balance of kinetic and thermodynamic processes, as shown in the following electrochemical reactions.
Figure 2.3.1. (A) Schematics describing the steps of nanoparticle substrate preparation via a galvanic electroless process of silver, Ag, on a 4.5 nm thick germanium, Ge, evaporated on a quartz substrate. Top surface AFM images of (B) quartz, (C) germanium on quartz, and (D) Silver/germanium on quartz are shown. From: Langmuir 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.

\[
\text{Ge} + 4\text{Ag}^+ \rightarrow \text{Ge}^{4+} + 4\text{Ag}, \quad \text{(Eq. 2.3.2)}
\]

\[
\text{Ge}^{4+} + x\text{H}_2\text{O} \rightarrow [\text{GeO}_x\text{H}_y(aq)]^{(4-n)} + n\text{H}^+ \quad \text{(Eq. 2.3.3)}
\]

The overall thermodynamics are driven by the galvanic displacement, with the kinetics and spatial (topography) aspects of the process controlled by charge transport. While the exact details of Equation 2.3.3 remain unclear, after nucleation nanoparticle growth follows by diffusion of \text{Ag}^+ from the solution to the nanoparticle surface ensued by adsorption and electron transfer from germanium substrate atoms through the nanoparticle to reduce the adsorbate ions. The simultaneous oxidation process is
driven by the donation of electrons from the germanium atoms. The final thermodynamic state is reached by solvation of germanium (IV) surface species with water to form soluble GeOₓHᵧ species which undergo dissolution, thereby leaving fresh germanium film surface for subsequent reaction.

As in every chemical transformation, the concentration of reacting species plays a crucial role in the kinetics; in this particular process, the silver reduction is strongly influenced by the concentration of Ag⁺ in solution. Therefore, among all the steps described previously, Ag⁺ diffusion to the growing nanoparticle surface is inferred to be the rate limiting one for the conditions we employed in this study. It is important to state that for the short time exposure to the Ag⁺ solution, the germanium substrate film is neither depleted nor does the amount of remaining germanium limit the reaction rate. The presence of germanium after nanoparticle deposition is established by the observation of a characteristic germanium peak at 280 nm in the optical absorption as displayed in Figure 2.3.2(A) and the Ge(L) peak at 1.2 eV in the energy-dispersive X-ray spectroscopy (EDS) spectra of the sample, which is shown in Figure 2.3.2(B).

In order to understand the responsive release of silver nanoparticles, we meticulously characterized the germanium nanoposts after removal of the silver nanoparticles from the germanium surface. We utilized octadecanethiol (ODT), which acts as a surfactant to detach the nanoparticle by forming hydrophobic self-assembled monolayers on the silver⁷² and germanium⁷³ surfaces without removing the underlying
germanium layer. Additionally, ODT does not form a monolayer on sidewalls of the nanoposts, which are protected by the germanium oxide layer. This nanoparticle detaching step was carried out as follows, the silver/germanium nanofilms were individually immersed in 20 mL of the 5 mM ODT solution, in anhydrous ethanol, for 24 hours for complete removal of the silver nanoparticles. Then, the silver/germanium films were mildly sonicated in anhydrous ethanol for 2 minutes; after sonication, the samples were washed with anhydrous ethanol and dried under a stream of nitrogen.

![Figure 2.3.2](image1.png)

**Figure 2.3.2.** (A) Extinction and (B) EDS spectrum of silver/germanium substrate. The EDS spectrum is collected after ODT wash, which removes the silver nanoparticles from the surface. The instrument used for EDS acquisition consisted of a Philip XL-30 scanning electron microscope (SEM) equipped with an Ametex attachment; the SEM was operated at an accelerating voltage of 5 kV to minimize sample damage. From: *Langmuir* **2012**, **28** (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.

The AFM image in Figure 2.3.3(A) shows the bare germanium posts – created by the galvanic displacement of Ag⁺ on germanium substrate for 30 seconds exposure to Silver nitrate solution – after the ODT-driven detach process. Careful analysis of AFM
images for several immersion times shows a nonlinear time dependence of the average post height (or adjacent crater depth), as described in Figure 2.3.3(B) with an integrated average of ~0.15 nm/s. Assuming that the dissolution of GeOxHy is not rate limiting, then the growth is controlled by the diffusion of Ag⁺ to the metal nanoparticle surface. Thus, for a constant Ag⁺ concentration gradient in the vicinity of the nanoparticle surface and considering a spherical nanoparticle, the rate of increase in the nanoparticle volume should be proportional to its surface area as \( \frac{dR_{NP}}{dt} \propto R_{NP}^2 \), where \( R_{NP} \) is the radius of the nanoparticle, and \( \frac{dR_{NP}}{dt} \) is constant, meaning that the \( R_{NP} \) increases linearly with time, \( t \).

**Figure 2.3.3.** (A) AFM image of the germanium surface (0.5 x 0.5 micron) which clearly shows the nanoposts structure that forms after 30s immersion in a 2 mM silver nitrate solution. AFM image is recorded after removing the silver nanoparticles from the surface by ODT surfactant. (B) Etch depth of the germanium surface at various times of the nanoparticle synthesis. The red curve shows a trendline, approximated to \( t^{1/2} \), for the etching rate of germanium. From: *Langmuir* 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.
If we look back at the charge balance in the redox equation (Equations 2.1 and 2.2), it is required that the amount of germanium lost from the surface proportionally relates to the amount of silver reduced. Therefore, the depth of germanium etched or equivalently the pillar height must also be linear with time, considering a hemispherical etch profile for the germanium film previously shown in figure 2.3.1(C). On the other hand, figure 2.3.3(B) shows a sublinear dependence that is explained by a decrease of the Ag⁺ concentration gradient with time due to rapid consumption of Ag⁺, which cannot be compensated by diffusion. This interpretation is consistent with Ag⁺ diffusion being the rate limiting step.

**Figure 2.3.4.** (A) Extinction spectra of silver nanoparticles on germanium film (black) in air and silver nanoparticles released to the acetic acid solution (pH = 6.0) after 2 seconds (dots) and 800 seconds (dashes) of immersion. Inset spectra show the extinction of released silver nanoparticles in time series (2, 100, 200, 400, 600, and 800 seconds after the immersion). The progress of the acquired spectra is shown in time from low to high extinction. (B) Time series spectra showing the absorbance of silver nanoparticles released into a tris(hydroxymethyl)aminomethane (Tris) buffer solution (pH = 8.0). Spectra are shown for 2, 4, 10, 20, 50, 100, 150, 200, 300, 400, 500, and 600 seconds (time progressing from low to high extinction) after immersion into the buffer solution. Illustration in the inset describes the optical configuration employed for monitoring the released nanoparticles (plane view). From: *Langmuir* 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.
As seen in Figure 2.3.4(A), the deposited film of immobilized silver nanoparticles exhibits an optical extinction spectrum consisting of quadrupolar and dipolar surface plasmon resonances peaking at 362 and 435 nm, respectively. The dipolar plasmon peak further shows a broad shoulder band 500 to 850 nm that we ascribe to plasmon hybridization due to dipole coupling between adjacent particles; since the frequency of the hybrid plasmon mode varies with interparticle spacing, we explain the extensive broadening in this shoulder band by the distribution and aggregation states. The low-intensity extinction spectrum in Figure 2.3.4(A) is associated with a negligible release of silver nanoparticles after the substrate is immersed in a solution of acetic acid (pH = 6.0) for 2 and 800 seconds upon immersion. A negligible fraction of the nanoparticles is released upon immersion in pH = 6.0 solution. Indeed, no remarkable change occurs in the appearance of the substrate. The inset shows a smaller region of the spectra. The extinction saturates at 800 seconds, beyond which further release is not measurable. We infer about half of the release happens in 2 seconds, that is immediately after the immersion. Therefore, the release must be due to weakly attached nanoparticles. Likely, these are larger nanoparticles as implied by the red-shifted resonance band. Similar results are obtained upon immersion in de-ionized water (pH = 6.5). The inset of Figure 2.3.4(B) illustrates the experimental setup employed for the time-lapsed extinction spectral data acquisition. Basically, the spectra are acquired by transmitting the optical beam through the solution only and excluding the nanoparticles immobilized on the substrate. The time-lapse extinction measurements are performed by immobilizing a 1×3 cm² sample in a 1×4x1 cm³ quartz
optical cell; then, the acquisition of time series spectra at 2 seconds intervals is initiated and followed by sudden injection of 3 mL of solution into the cell. The solution is stirred with a magnetic stirrer located at the base of the cell at a speed of 60 rpm (optical beam passes 1.5 mm above the base of the cell). Thereby, we can selectively monitor the released particles in real time.

In Figure 2.3.4(B) the active nanoparticle release is evidenced by the increase in the extinction intensity of the LSP peak of the nanoparticles; however, no discernible change in the shape of the spectrum is observed in time. This interesting finding indicates that the distribution of monodisperse and aggregate nanoparticle populations remain the same, suggesting that the released particle aggregation must occur shortly after the nanoparticle release. In addition, it is extremely likely that the nanoparticles start aggregating on the vicinity of the germanium substrate surface, where the nanoparticle density is the highest and consequently the aggregation rate as well. This study serves conclusive corroborating evidence that the developed nanofilms can exhibit a spontaneous and autonomous response (release of silver nanoparticles) after being triggered with a specific stimulus.

### 2.4 Protein Biomarker Detection

The developed stimuli-responsive nanoparticle film was initially tested for the detection of a biomarker protein. More in detail, we focused on detection of recombinant
lectins, which are carbohydrate binding proteins, using SERS as the detection technique.

The recombinant lectin protein that is employed in this study is developed from *Aleuria Aurantia lectin* (AAL), a mushroom-derived lectin that preferentially binds to fucose linked (α-1,6) to N-acetylglucosamine but also binds to fucose linked (α-1,3, α-1,2, α-1,4) to N-acetyllactosamine related structures. In this exploratory study we were interested in the detection of L-fucose, as fucosylated molecules are predictive of hepatocellular carcinoma (HCC) and other liver diseases.

Figure 2.4.1 shows the crystal structural data, indicating that AAL has 5 (genetically distinct) fucose binding sites with each site potentially having unique binding affinities for different fucopyranosyl linkages. However, the lack of fucopyranosyl linkage specificity in native AAL represents a problem for specific, and high-affinity detection.
experiments. Dr. Patrick Romano, a collaborator at Hepatitis Research Institute, has produced and purified a recombinant form of AAL (rAAL), which has higher fucose binding affinity than the natural product. Romano and collaborators synthesized a DNA encoding the AAL gene sequence modified to include a C-terminal histidine tag followed by two cysteine residues; which provides a higher affinity to fucose-linked moieties. Additionally, terminal cysteine functionalities of the rAAL protein facilitate the binding to the silver nanoparticles. Using standard molecular biology cloning and expression techniques, the sequence was expressed from a T7 inducible pET vector in a BL21 (D3) bacterial strain. Expression of rAAL was induced using 0.5 mM isopropyl β-D-1-thiogalactopyranoside. Cells were harvested, lysed and the rAAL was purified using fast protein liquid chromatography immobilized metal affinity chromatography. The purity of rAAL was verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis followed by coomassie staining.

Protein detection studies were performed by adding 1 µL of 1µM rAAL, in a phosphate buffer solution (PBS, pH 7.25), onto the silver/germanium nanostructured films (prepared as described in section 2.3 of this chapter) and SERS spectra was recorded 1 minute after addition; the pH of the solution is intentionally kept in an alkaline region to trigger particle responsive release. In addition, target binding studies were performed using L-fucose as a target sugar. Previously, the signature spectrum for the L-fucose sugar was recorded. In the target assay, 10 µL of a 100 µM L-fucose solution in PBS (pH 7.25) was mixed with 10µL of AAL and incubated at 3 °C under constant stirring for 3 hours; target binding detection was accomplished using a 1 µL aliquot from
the L-fucose – rAAL incubating solution. SERS spectra were recorded using a Renishaw inVia micro-Raman instrument equipped with a HeNe laser source (633 nm), a motorized microscope stage, and a CCD detector. The acquisition parameters were 20X objective 10 second acquisition time at 250 µW and plotted using Renishaw software without any averaging or smoothing (i.e. raw data). To test the stability and photometric reproducibility of the instrumental setup, we measured the uniformity of the silicon phonon peak across a 40 mm² area of a highly uniform single crystal silicon substrate. For all the samples, the SERS measurements were recorded in solution, in order to get uniform spectra with minimal spot-to-spot signal variation.

It should be noted that the concentration of L-fucose target sugar is considerably higher compared to the concentration of rAAL. This target excess was intentionally implemented to assure that the L-fucose sugar targets each binding site of the rAAL protein. Figure 2.4.2 shows the SERS spectra recorded at 633 nm for the rAAL, and rAAL-L-fucose. L-fucose sugar has characteristic resonance bands at νC-O 1393 cm⁻¹ and νC-H 963 cm⁻¹, and the νC-O-C antisymmetric stretch at 1223 cm⁻¹. SERS spectrum of rAAL shows vibration bands at νC-H 1324 cm⁻¹, νAmide III 1262 cm⁻¹, νPhe (1160 and 923 cm⁻¹), νC-N 1003 cm⁻¹, νTyr 942 cm⁻¹. For the target assay consisting of rAAL with L-fucose, a distinguishing peak is observed at 1198 cm⁻¹, which is ascribed to the C-O-C antisymmetric stretch of the fucose molecule. A slight shift of the Raman peak, νC-O-C, between the bare fucose (1223 cm⁻¹) and lectin bound fucose (1198 cm⁻¹) can be attributed to binding between the fucose and lectin. It should be remarked that this is the only peak from the L-Fucose SERS signature spectra that is observed in the
target bioassay, which is an unambiguous indication of the binding interaction between the rAAL protein and the target, as L-fucose does not possess any binding site that can facilitate the adsorption or binding to the nanoparticle surface.

This study serves as a proof of concept for the developed plasmonic sensor array, allowing the rapid detection of biomarkers in solution. The study establishes the basis for the execution of more complex experiments aimed at sensing and molecular recognition.

| Table 2.4.1. SERS peak assignments for L-fucose and rAAL |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| L-Fucose Peak (cm⁻¹) | L-Fucose Peak Assignment | r-AAL Peak (cm⁻¹) | r-AAL Peak Assignment |
| 1391 | νC=O stretching deformation | 1324 | νC-H δ(CH) stretching |
| 1223 | νC-O-C asymmetric stretching | 1262 | νAmine III stretching |
| 963 | νC-H deformation | 1160 | νPhe δ(CH) deformation |
| 1003 |  |  | νC-N Stretching |
| 942 |  |  | νPhe Stretching |
| 923 |  |  | νTyr Stretching |
Figure 2.4.2. SERS spectra recorded at 633 nm of (black) the recombinant versions of AAL, (blue) recombinant version of AAL with L-fucose, and (red) L-fucose. Reproduced with permission of the IEEE copyright 2011.

2.5 Detection of Viral Molecular Probes

After completing a concept study of the developed stimuli-responsive nanoparticle arrays with a protein biomarker, we engaged in more complex studies aimed at the specific detection of molecular probes (MP) pertaining to target virus like hepatitis B virus (HBV) and respiratory syncytial virus (RSV). As part of this work, the silver/germanium nanostructured thin film is patterned into high-density array chips with specific MPs at each pixel for an accurate and reagentless screening of multiple nucleic acid targets (i.e. HBV and RSV) in a single test. A schematic depiction of this process
is shown in figure 2.5.1(A), accompanied by a microscope image of the silver/germanium patterned nanoparticle layer as shown in figure 2.5.1(B).

As part of this study, DNA probe sequences from the RSV and HBV genome were selected. These sequences were evaluated for minimal secondary structures, loop and dimer formation using Gene Runner DNA analysis software (free download available at www.genelink.com). The nearest neighbor $T_m$ of the probes is $60\,^\circ C$. A hairpin sequence was added to the 5’ and 3’ end of the sequence of each probe, which yields a stable hairpin structure without creating a loop and secondary structure with the target loop sequence. The DNA MP sequences were synthesized at Gene Link with a 3’-thiol and a 5’-fluorophores (i.e., Cy5) containing sequence as detailed in Table 2.5.1. The probe was synthesized using 3’ thiol C6 CPG (Glen Research, VI, USA) as the solid support followed by the oligo sequence and coupled to Cy5 dye. The crude probes were obtained after complete deprotection were ethanol precipitated and polyacrylamide gel purified. Immobilization of DNA, unless otherwise stated, was carried out as follows: Probe-DNAs with different concentrations ($10^{-6} - 10^{-10}$ M) in dimethyl sulfoxide solution were incubated on the silver/germanium surface for 10 minutes at room temperature. After the conjugation of the MPs, surfaces were washed several times with dimethyl sulfoxide solution and dried under a nitrogen stream.

Hybridization studies were carried out by adding a 1 µL of complementary DNA (at least 4-fold of probe DNA concentration) onto the silver/germanium surfaces using a buffer solution (50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl$_2$ with a pH of 8.0). The
hybridization assay was allowed to react for 1 minute at room temperature. Figure 2.5.1(C) shows a high-resolution image describing this process; Figure 2.5.1(D) shows the transmission electron microscopy (TEM) image of a silver nanoparticle layer of one patterned chip after analyte-induced aggregation and drying.

**Table 2.5.1. Designed RSV and HBV molecular probes**

<table>
<thead>
<tr>
<th>Synthetic positive control template (SPCT) for RSV</th>
<th>TTTGTTGGTGTGGGATTGTT-GGCTCTTCTGTTGGCTTGGTG</th>
<th>G probe complementary in M11486: 5321-5360</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV G gene probe V</td>
<td>CGCAGCCACAGAAGAGCAAAC-CATCAACACTGCG</td>
<td>G probe position in M11486: 5329-5352</td>
</tr>
<tr>
<td>Synthetic positive control template (SPCT) for HBV</td>
<td>TCCTGGTTATCGCTG-GATGTGTCCTGGGCGTTTTATCATA TTCTTCTTCTCCTGCTG- TATGCCTCATCTTCTTATTGG-TACTTCTGGAT- TATCAAGGTATGGTTCGCTGGTT- GTCCTCTAATCCAGGAAC- CACAACACACCAGTGACGACCCTG-</td>
<td>ACC# GQ331048; position 361-420</td>
</tr>
<tr>
<td>HBV probe</td>
<td>CGTGGGCTCCCTTCATCCTGCTGCTC-TATGCTTCTCATCCTCCAG</td>
<td>Probe is specific to all (A-H) HBV Sub-types</td>
</tr>
</tbody>
</table>

As in our previous protein biomarker study, a Renishaw inVia microRaman instrument was used for the detection of DNA molecules on the substrates. The acquisition parameters were 20 X objective and 2 second acquisition time at 250 μW with a grating of 1800 lines/mm, and plotted using Renishaw software without any averaging or smoothing (i.e. raw data). In addition, a fixed silicon wafer was used as a reference for normalizing the variation of power in different scans. The SERS
measurements for DNA are collected on the drop, which is away from the silver/germanium substrate surface, as shown in Figure 2.5.1(A).

![Figure 2.5.1.](image)

**Figure 2.5.1.** (A) Schematic diagram describing steps of microarray preparation, molecular probe conjugation, hybridization of the target sequence, and detection. (B) Arrays of micro-patterned nanoparticle layer. (C) 1 µl droplet on one of the microarray spots is shown, (D) TEM image of the silver (Ag) nanoparticles after analyte induced aggregation and drying. From: *Langmuir* 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.

Typically, in a sensing or molecular recognition experiment, it is expected to observe a signal that confirms the presence of a given analyte. However, this study employs an indirect negative feedback mechanism, meaning that the presence of the target is confirmed by a decay of the SERS signal. The Cy5 fluorophore provides resonance Raman signal in the closed configuration of the MP because the stem hybrid keeps the fluorophore close to the nanoparticle layer. When the probe hybridizes to RSV or HBV
target, a conformational change occurs that separates the fluorophore from nanoparticles and the SERS signal vanishes.

**Figure 2.5.2.** Molecular probes attached to the responsive active release nanoparticle substrate based on the schematics described in Figure 2.5.1. (A) RSV and HBV gene detection is tested on a microarray format using a Renishaw Raman microscope. Raman signals show clearly distinct spectra of Cy5, especially around 1200 cm\(^{-1}\) and 1600 cm\(^{-1}\) which are the fingerprint peaks for Cy5 dye on the molecular probe. (B) The sensitivity of the RSV-Cy5 shows a detection limit of 100-1000 molecule. (C) Molecular probe specificity of RSV-MP tested using two target sequences (RSV and HBV) which are designed based on Table-2. RSV target results in loss of the SERS signal but HBV target shows Cy5 signal. (D) Schematic diagram illustrating the molecular probe cross-reactivity assay. Adaptation from: *Langmuir* 2012, 28 (14), 5975-5980. Reproduced with permission of the American Chemical Society copyright 2012.
Figure 2.5.2 summarizes the target nucleic acid detection of RSV and HBV probes and a control molecule. Figures 2.5.2(A) shows the SERS spectra of the Cy5 for RSV and HBV MPs. We have observed characteristic bands at $\nu_{C-N}$ 1271, $\nu_{\text{methine}}$ 1385 and $\nu_{C=N}$ 1594 cm$^{-1}$. The SERS spectra and peak locations match well with the literature, detailed summary of the peak assignments for the Cy5 MP is shown in table 2.5.2.$^{80}$ The detection limit for the developed nucleic acid biosensor is explored by placing various concentrations of the sample over the patterned substrate surface. Figure 2.5.2(B) shows the Raman signal count at 1594 cm$^{-1}$ peak for various concentrations of RSV MP. The data shows that the detection limit is reached at 0.1 nM concentration. This SERS biosensor platform obviates the need for target DNA amplification and provides a multiplexed target detection, at the same time. Detection limits obtained by this technology are competitive with the lowest values reported for electrochemical ($\sim$1000-3000 copies), fluorescent assays ($\sim$60,000 copies), mechanical ($\sim$10,000 copies) and other reported methods.$^{81}$

Additionally, the specificity of the developed SERS substrate is demonstrated by testing the cross-reactivity of RSV and HBV target sequences as illustrated in Figure 2.5.2(C-D). When the probe encounters the RSV gene, it forms a rigid double helix which is more stable than the stem (hairpin structure). Consequently, the MP undergoes a spontaneous conformational reorganization that forces the stem apart, causing the fluorophore to move away from the nanoparticle surface when hybridized, leading to the loss of the Raman signal. Therefore, when RSV probe hybridizes its target, it results in a complete loss of the Raman signal, but if it is exposed to the HBV target
(in the cross-reactivity test), the Cy5 signal remains as shown in Figure 2.5.2(C), which represents an unambiguous confirmation that HBV genes did not bind to the RSV probe.

Table 2.5.2. SERS peak assignment for Cy5 molecular probe

<table>
<thead>
<tr>
<th>Raman shifts</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1594 cm(^{-1})</td>
<td>(\nu_{\text{C=N}}) Stretching</td>
</tr>
<tr>
<td>1500 cm(^{-1})</td>
<td>Aromatic ring vibrations (\nu(\text{C-C})) or/and (\nu(\text{C=C}))</td>
</tr>
<tr>
<td>1400-1425 cm(^{-1})</td>
<td>(\delta(\text{CH2})) and (\delta(\text{CH3})) asymmetric stretching</td>
</tr>
<tr>
<td>1385 cm(^{-1})</td>
<td>(\nu_{\text{methine chain deformation}})</td>
</tr>
<tr>
<td>1271 cm(^{-1})</td>
<td>(\nu_{\text{C-N}}) stretching</td>
</tr>
<tr>
<td>1200 cm(^{-1})</td>
<td>(\nu_{\text{C-C}}) ring stretching</td>
</tr>
<tr>
<td>1150 cm(^{-1})</td>
<td>(\nu_{\text{C-H}}) beta</td>
</tr>
</tbody>
</table>

2.6 Summary

This chapter introduced the concept of surface plasmons and explained the difference between LSPs and SPPs, which accounts for different plasmonic processes. Herein, we reported the development of a thin-film nanoparticle array that provides a dynamic sensor platform based on pH-selective release of metal nanoparticles. The nanoparticle active release can be conceptualized as an autonomous response, which is triggered by a given stimulus (alkaline pH in this case), in analogy to smart materials. Furthermore, the surfactant-free nanoparticles exhibit direct and intimate plasmonic hybridization with the optical probe, which is not observed in nanoparticle systems synthesized from solution.
Our nanoparticle arrays are proposed as innovative thin film biosensors, in order to prove the concept, we executed two model experiments. One consisted of the detection of a protein biomarker, rAAL, that exhibit high affinity and specificity for L-fucose sugar, which can be employed as a predicting agent for HCC and other liver diseases. The second model study utilized metal nanoparticles pre-functionalized with nucleotide-specific molecular probe molecules, which report the presence of DNA using Raman spectroscopy as an optical probe.

The established sensor scheme can be integrated into low-cost, real-time diagnostic devices based on Raman spectroscopy and additional optical platforms (i.e. optical cavity resonators, nanoparticle-based SPR, etc.), for the presence of analytes at low concentration, avoiding the necessity of incurring into amplificatory techniques. The integration of cross-disciplinary technology advancements has the potential to improve the detection capabilities of our biosensor for all facets of biomarkers and gene detection, including single nucleotide polymorphism, gene expression, mutation analysis, quantitative detection, as well as environmental agents and small molecules, including routine laboratory tests for biochemical agents like cholesterol, uric acid, creatinine, and proteins (enzymes). In summary, the stimuli-responsive release of nanoparticles arises as an extremely versatile methodology, capable of being extended to a number of other detection applications due in part to its inherent potential to be integrated into different optical probes. However, despite these advantages, there is a standing necessity to develop novel platforms that allow the analyte detection at ultra-low concentrations in real-time.
Chapter 3

Optical Resonances and Confined Systems: An Introduction to Whispering Gallery Systems**


3.1 General Description of Resonator Systems

The word resonance is used to describe a closed and periodic trajectory that is followed by an object over a period of time. A classic example of a system exhibiting a resonance is a pendulum clock. Looking more closely at this resonant system, it can be realized that the pendulum is one of the first energy converters. The total energy of a system is given by its potential energy (associated with position), plus its kinetic energy (associated with movement). A pendulum system reaches its highest potential energy or – maximum stored energy – when located at the highest from its midpoint; as the pendulum accelerates towards its midpoint the potential energy is converted to kinetic energy, then back to potential energy during the climbing. In this way, the
pendulum is one of the finest examples to conceptualize the conservation of energy in a macroscopic physical system. Although it should be noted that part of the energy of the pendulum is often lost due to air resistance. As a consequence of air friction, the pendulum covers a shorter distance; however, it does it more slowly, taking actually the exact same time to swing. This ability is called isochronism, meaning equal amounts of time, and that is the reason why pendulums have been used as timekeeping devices.

As mentioned above, resonances are extensively applied in different scientific fields, and researchers have exploited resonances to probe the behavior of materials, in attempts to elucidate their chemical identity as well as fundamental properties of their molecular constituents. In optics, resonances account for the movement of photons exhibiting a periodic trajectory among a defined number of points. An optical resonator is a structure able to confine light waves in small volumes, generating an evanescent field as a consequence. Resonating cavities in optics have been intensively utilized as optical gain mediums in laser systems, molecular detection, and molecular manipulation. 

The term microcavity is utilized to define a miniaturized structure consisting of reflecting faces that accumulates light at high field intensities in a relatively small volume. The confinement of light in such restricted scales generates an evanescent field that is extremely sensitive to external perturbations such as the presence of a foreign element.
Typically, resonances propagating within an optical resonator can be observed as dips in a transmission or reflection spectra. The resolution of an optical resonator is defined in terms of its ability to accumulate light at a given frequency, also referred as photonic lifetime or quality factor ($Q$):

$$Q = \frac{\lambda_0}{\gamma_0}$$  \hspace{1cm} (Eq. 3.1.1)

Where, $\lambda_0$ is the wavelength of the resonance peak, and $\gamma_0$ is the linewidth, which is defined as the full width at the half maximum (FWHM) of the resonance peak.

One of the earliest resonators utilized as gain mediums in cavity lasers was the Fabry-Perot cavity (FPC), which consists of two reflectors spaced from micrometers to centimeters apart. Typically, the more reduced the mirror separation is, the more optical field FPCs are able to confine. FPCs have been widely employed in interferometric processes, communications, and proposed as sensing platforms. However, one of the most prevalent challenges that these structures have faced in the sensing field is associated with their ability to confine light, which drastically affects the resolution of these systems; although high-quality mirrors can be fabricated, the elaboration of such mirrors is laborious and expensive, limiting their utilization as sensors in a practical scenario.
Progress in the synthesis and manipulation of materials have enabled the development of photonic crystals (PCs), which are periodic dielectric nanostructures that selectively couple certain frequencies – or wavelengths – of light while excluding others. Depending on the periodicity and refractive indexes of the dielectric materials forming the PC structure, light can be manipulated in exotic ways. In this specific case, a two-dimensional photonic crystal slab confines an optical field in the $Z$ direction due to the contrast between the slab and the surrounding substrate and air, and the periodicity from the patterns confine the optical field in the $X$ and $Y$ directions. Although relatively good resolution and sensitivity can be obtained with these structures, the fabrication of perfect periodic dielectrics is extremely challenging and expensive.

The numerous applications attributed to optical resonances provides a fertile ground for further exploration. The following sections of this chapter are devoted to optical microcavity sensors that accumulate high field intensities in the form of confined electromagnetic resonances, and the combination of these cavities with plasmonic nanoparticles like gold in order to enhance their sensitivity without degrading their resolution.
3.2 Optical Microcavities and Whispering Gallery Resonances

Recently, a new class of optical resonators has become a novel approach to accumulate light at high field intensities, as evidenced by their ultra-high Q-factors. The propagating resonance wave is coined a “whispering gallery” (WG) mode as an analogy to the sound-guiding effect described by Lord Rayleigh in 1910. This phenomenon was studied in the hemispherical dome of Saint Paul’s Cathedral in London. Two people facing the wall would be able to communicate even at a whisper. However, they would not be able to hear each other if trying to communicate across the center of the room. The WG effect is caused by the guided propagation of sound waves along the smooth and curved walls of the periphery of the dome, which minimizes scattering and dissipation as compared to a sound wave trying to travel across the center of the dome.

Figure 3.2.1. Schematic depiction describing the constructive (A) and destructive (B) interference path on the cross-sectional plane of a WG optical cavity.
Optical microcavities – also referred as WG resonators – are able to support electromagnetic resonances through the confinement of internally reflected waves within the microresonator structure. The resonance condition is given by Eq. 3.2.1:

\[ m\lambda = 2\pi R n_{eff} \]  \hspace{1cm} \text{Eq. 3.2.1}

The confinement of a resonant field with effective refractive index \( n_{eff} \), and within a resonator of radius \( R \) occurs if the scalar \( m \) is an integer at the excitation wavelength \( \lambda \). The importance of Equation 3.2.1 is that it dictates the principle for confining resonance modes inside a microcavity resonator, which results in the excitation of WG modes due to constructive interference of resonant electromagnetic waves. Figure 3.2.1 facilitates the illustration of this concept by representing the cross-sectional area of a resonator exhibiting an “on resonance” path (A), where \( m \) is an integer, and an “off resonance” path (B), where \( m \) is not an integer.

In the optical microcavities utilized in this research, light is channeled through a guiding structure called a waveguide; Figure 3.2.2 describes this process. Typically, single mode optical fibers – at the guiding wavelength – are selected for this purpose as they are carefully designed to carry the transverse mode of electromagnetic radiation, meaning that the output of the fiber is a single ray of light.
**Figure 3.2.2.** Schematic diagram describing the planar view of an optical WG microresonator. A tunable laser is employed to send electromagnetic waves to a photodetector, and an optical fiber is used to guide that light into its final destination. The thinned (tapered) region of the fiber generates an optical field that evanescently propagates into the medium. WG modes are excited by confining the evanescent waves of the fiber inside the resonator structure, which is accomplished when the microcavity is brought into mechanical contact with the fiber. The excitation of WG modes is evidenced as dips in the transmission signal captured by the photodetector.

Electromagnetic waves traveling through an optical fiber are contained within a protective material, also referred to as cladding, which is surrounded by a polymer material that protect the fiber from breaking, also termed as polymer jacket. The polymer jacket can be selectively removed, exposing the cladding material containing the fiber to the environment; then, the fiber is subsequently tapered by stretching it under the thermal influence of a high-temperature flame (i.e. hydrogen, or oxygen-propane), a microheater, or a CO₂ laser. The tapering process exposes the fiber core and generates an evanescent field that propagates into the surrounding medium. At this point, the microresonator cavity can be placed in mechanical contact with the thinned tapered fiber and the fiber-resonator contact region can be illuminated with the evanescent waves from the fiber. Similarly, structures like optical fibers or prisms can be
utilized to drive optical resonant modes inside a dielectric resonator (i.e. a micro-
sphere). However, there are some advantages associated with the use of optical
fibers in biosensing. Namely, the simplicity of exciting WG modes, ease of resonator-
fiber alignment, and the possibility of using a new waveguide, and a new resonator
for each experiment, avoiding the need for cleaning procedures.

The optical field stored in a WG mode can be described by a Lorentzian line shape
defined by expressing the power ($P$) as a function of the wavelength:

$$P(\lambda) = P_r \frac{\left(\frac{\nu}{2}\right)^2}{(\lambda-\lambda_0)^2+(\frac{\nu}{2})^2}$$  \hspace{1cm} (Eq. 3.2.2)

Where $P_r$ denotes the amplitude of the confined wave. The excitation of WG modes
cannot be directly observed from the $P(\lambda)$ function since the energy is trapped within
the microresonator cavity. Experimentally, the excitation of WG modes can be in-
ferred by monitoring the transmitted power as a function of the illumination wave-
length, $P_t(\lambda)$ as shown in Figure 3.2.2. The minima in the transmission signal corre-
sponds to electromagnetic waves resonating within the microcavity structure and
have a characteristic Lorentzian shape that is dependent on the coupling efficiency,$\beta$, and related to the input laser power, $P_0$, as defined by:

$$P_t(\lambda) = P_0 - \beta P(\lambda)$$  \hspace{1cm} (Eq. 3.2.3)
The optical confinement in WG resonators is driven by total internal reflection (TIR), which occurs when two conditions are met: (i) light must propagate from an optically denser medium with refractive index $n_1$ into a less dense medium with refractive index $n_2$, such that the following relation can be established $n_1 > n_2$; (ii) the angle of incidence of the optical wave ($\theta_i$) must be greater than the critical angle ($\theta_c$), thus $\theta_i > \theta_c$. By definition, $\theta_c$ denotes the angle at which TIR occurs, which can be calculated from Snell’s law, $n_1 \cdot \theta_c = n_2 \cdot \theta_n$, by setting the reflection angle ($\theta_r$) as $\pi/2$.

In theory, WG resonators should be able to confine electromagnetic waves that propagate according to the selection criterion dictated in Equation 3.1.1 ad infinitum. However, real-life divergence, in other words, material’s imperfections and inhomogeneities induce optical losses, which are accounted for by the cavity Q-factor as described in Equation 3.1.1. As there exist different channels such as radiation, surface and bulk scattering, as well as material absorption that influence optical losses, the FWHM ($\gamma_0$) can be expressed as the addition of these individual contributions $\gamma_0 = \gamma_{\text{rad}} + \gamma_{\text{sca}} + \gamma_{\text{mat}} + ...$; therefore, there are three main factors – fabrication quality, intrinsic material properties, and resonator size – which determine the ability to confine light of a resonator (Q-factor). For instance, in a small resonator radiative losses dominate; when the microcavity radius is increased, a larger volume is available to confine optical modes through constructive interference, minimizing – in this way – radiative losses, which yields a higher Q-factor. Therefore, radiation loss is dependent on the resonator size and can be attenuated by increasing the diameter of the resonator. However,
as the resonator size is further increased above a certain value dictated by the geometry of the resonant microcavity; optical absorption, as well as internal material’s defects, govern the optical losses, and the Q-factor is dominated by the absorption coefficient \( a \) as shown in Equation 3.2.3:

\[
Q = \frac{2\pi n_r}{a\lambda}
\]  

Eq. 3.2.4

Where \( n_r \) accounts for the refractive index of the resonator. Alternatively, the Q-factor of a microcavity resonator can be optimized by carefully selecting the illumination wavelength. The smaller the illumination wavelength is, the more waves are being placed into the optical microcavity, which translates into a higher number of resonances, and subsequently a larger Q-factor.

In order to determine the sensitivity of a resonator, we can assume that the smallest detectable wavelength displacement \( \Delta\lambda_{\text{min}} \) should be in the order of the resonance linewidth \( (\gamma_0) \). Thus Equation 3.1.1 can be rewritten as:

\[
\Delta\lambda_{\text{min}} = \gamma_0 = \frac{\lambda_0}{Q}
\]  

Eq.3.2.5

Therefore, as the resolution increases, the smaller \( \Delta\lambda_{\text{min}} \) becomes, which results in a higher sensitivity.
The light intensity within the resonator structure is given by the volume that a resonant WG mode occupies considering a homogeneous energy distribution, as described by Srinivansan et al.\textsuperscript{89}:

\[ V = \frac{\int n_r^2(r)|E(r)|^2 \, d^3r}{n_m^2(r)|E(r)|^2} \quad \text{Eq. 3.2.6} \]

Where \( V \) denotes the mode volume, \( n_m \) is the refractive index of the medium, and \( |E(r)|^2 \) is the electric field value at a position \( r \). The modal volume of a WG cavity influences the light intensity inside the resonator, and therefore the strength of light-matter interactions. Smaller \( V \), implies a more enclosed field; for instance, as the diameter of the WG microcavity is reduced, the optical modes are more confined, which translates into higher light intensities.\textsuperscript{90}

WG microresonators derive their sensitivity from their inherent ability to sustain high-Q resonances within the microcavity structure. When a foreign analyte with a different refractive index than the medium and the WG microcavity approaches the active surface of the resonator (the surface where the WG mode is excited), the effective surface of the resonator changes, resulting in a wavelength displacement. The molecular perturbation of a WG mode in terms of wavelength shift can be accurately predicted in the frame of the first order perturbation theory established by Arnold and Terakota.\textsuperscript{91-92}
\[ \frac{\Delta \lambda}{\lambda} = \frac{\alpha_p \sigma}{\varepsilon_0 (n_r^2 - n_m^2) R} \quad \text{Eq. 3.2.7} \]

In this particular case for a spherical resonator of radius \( R \), the fractional wavelength displacement \( (\Delta \lambda/\lambda) \) of a WG resonance mode perturbed by an analyte with polarizability \( \alpha_p \), can be related to the surface area of the analyte \( (\sigma) \).

WG microcavities have exhibited unprecedented resolution, which is derived from their ability to confine high-intensity resonant fields (Q-factor up to \( 10^9 \)). The first observation of WG mode reported in in optics correspond to the one described by Garret et al, in which laser excitation was studied in a Sm:CaF\(_2\) crystalline millimeter-sized resonators. Properties of WG modes such as high resolution, sensitivity of the resonator’s active surface, and environment stability have made this technique well suited for diverse applications such as telecommunications and sensing. In the telecommunication research field, for example, the reduction in the required pump power and fiber coupling makes WG microcavities an excellent alternative for the development of microlasers, other applications in this field include the development of narrow filters, optical switching and molecular electronics. Similarly, WG microcavities have found a robust niche in the biodetection and sensors research field because of their superior sensitivity, as well as their ability to detect foreign analytes in real-time. They are ideal for fundamental studies of kinetics or intermolecular interactions in a label-free manner. In addition to the aforementioned applications, WG mode cavities can enhance for scintillators outputs: 68-72 % luminescence enhance-
ment has been observed relative to planar output methodology. Numerical calculations on solar cells have suggested that bottle-like and cylindrical resonators should provide a 30% improvement in power conversion efficiency when compared to a homologous planar cell.

Recent progress in fabrication techniques has permitted the proposition of alternate shapes and materials as WG microcavities. Geometries including microspheres, disks, rings, toroids, or cylinders have been developed. For a long period of time, WG resonator materials were limited to silicon dioxide, and other crystalline materials; recently, boundaries in material composition have been overcome with the development of soft-matter WG resonators.

3.3 Hybrid Photonic-Plasmonic Microcavity Whispering Gallery Resonators

In the previous section, the concept of WG resonators was introduced, as well as the strengths of this technology. However, there are some limitations associated with the ability to detect analytes at ultralow concentrations without amplification mechanisms. Generally, the detection limit can be quantified by the ratio of $Q/V$, and can also be expressed in terms of minimum detectable wavelength as explained, $\lambda_0 / Q$, as explained in Equation 3.2.5. Hence, to increase the sensitivity of a cavity it is necessary to increase the Q factor while reducing the mode volume. If we look at this in
more detail, we can refer to Equation 3.2.7, which dictates how the single Lorentzian resonant mode is perturbed by a foreign analyte. As shown in Equation 3.2.7 it can be established that there are two factors that profoundly affect the magnitude of the wavelength displacement; one factor is the polarizability of the analyte at a given wavelength, whereas the other one is its surface area. The polarizability parameter can be optimized – to some degree – by tuning the probing wavelength; regrettably, the only manner to optimize the surface area of the analyte bound to the resonator is to increase the concentration of the analyte. For small analytes with a size much smaller than the wavelength, it is difficult to decouple the displacement of the resonance signal from naturally occurring (i.e. thermal effects) in an ultrasensitive manner. Thus, it is important to explore novel ways to enhance the resonance field of the WG microcavity, such that ultrasensitive detection of analytes can be accomplished.

Several routes have been studied to accumulate the optical field in the resonator structure. For example, rare-earth dopants have enhanced the Q-factor of the WG resonances by providing additional channels of optical gains; in this approach, optical resonators have been doped with rare-earth ions such as erbium or ytterbium (Er$^{3+}$ and Yb$^{3+}$ respectively).\textsuperscript{110-114} In the optics community, such resonators are termed active resonators. The gain in detection sensitivity of an active resonator is accomplished by exciting beyond the lasing threshold. This particular finding has enabled the detection of particles in a highly sensitive manner.\textsuperscript{111-112,115-116} The active resonator approach has made a notable contribution in terms of sensitivity gain compared
to conventional WG mode systems. Regrettably, active materials require the utilization of specialized, laborious and expensive preparation techniques in order to achieve desired results; moreover, rare-earth dopants are incompatible with biomolecules, which limits its application to biodetection. Hence, there is demand for novel materials that are compatible with biomolecules and can enhance the sensitivity of current WG optical systems.

To overcome current limitations, we have designed a hybrid photonic-plasmonic WG platform. In this particular alternative, high-Q WG microcavities are combined with gold nanoparticle arrays in order to improve the sensitivity in WG mode systems. The inclusion of gold nanoparticles in combination with high-field WG resonances provides a novel alternative to intensify the resonant field through the excitation of LSPs while confining it to the nanoparticle surface. It should be noted, that noble metal nanoparticles like gold or silver a totally biocompatible, and different binding or anchoring routes have been established through ionic and metal-ligand interactions between the surfactant layer and the analyte of interest at standard synthetic conditions (i.e. without excessive heating nor complex chemical protocols). The next section of the chapter details this hybrid photonic-plasmonic concept, and experimentally describes the combination of WG with gold nanoparticles for the detection of a model protein that has been previously adsorbed on the nanoparticle layer.
3.4 Protein-Particle detection with microcavity resonators

The combination of WG microcavities and nanoparticle-based biosensing is extremely appealing: in principle, it should permit enhanced shifts in resonance signal upon analyte-particle conjugation, resulting in a higher sensitivity due to the coupling between WG resonances and Plasmon resonances. At the same time, careful considerations, detailed in the Microcavity-Particle Coupling section of this chapter (section 3.5), should be taken to minimize radiative losses.

Here, we demonstrate such a plasmonic nanoparticle-based detection methodology with label-free WG mode readout. In this approach, as illustrated in Figure 3.4.1(A), we use ~55 nm gold nanoparticles to unspecifically bind a model protein from a buffered aqueous solution. The nanoparticles are then filtered, immobilized and dried on an anodic aluminum oxide (AAO) capture-membrane. Subsequently, the nanoparticle layer is interrogated with WG modes from a microspherical cavity, and the wavelength displacement of the high-Q WG resonant mode is measured by repetitive evanescent coupling of the microcavity to – a part of – the nanoparticle layer. A dose response curve for the binding of the model protein to the nanoparticles is extracted from the WG mode resonance wavelength shift measured for different BSA solution concentrations.
Gold nanoparticles (~55 cm in diameter) were synthesized following a modification of the Lee and Meisel’s method\textsuperscript{55} similar to the one described by Kruszewski et al.\textsuperscript{56} In this particular case, gold colloids were prepared by a reduction of hydrogen tetrachloroaurate with sodium citrate; 100 mL of a $10^{-3}$ M hydrogen tetrachloroaurate solution was heated to 100 °C under constant stirring; at the boiling point, 2 mL of a 1% (weight by weight) trisodium citrate solution was added to the reacting mixture. Two minutes after the addition of trisodium citrate, it was observed that the citrate the reacting mixture turned into a gray color; the reaction was refluxed for 1 hour, finally
exhibiting a deep red color. At this point, the reaction was stopped and allowed to cool down at room temperature. Then, the synthesized gold colloids were incubated with bovine serum albumin (BSA) in a PBS solution. In this step, gold nanoparticles were diluted to a 1:1 (volume by volume) with ultrafiltered deionized water (18.1 MΩ cm). The diluted gold nanoparticles colloids were mixed with BSA at a 1:1 (volume by volume) ratio and incubated at 4 °C for at least 10 minutes; typically, solutions were used within 45 mins after incubation, but witness solutions have been stable for 4 hours at 4 °C. Solutions were prepared with concentrations ranging from pM to µM (0.01-, 0.1-, 10- and 10^4 nM) were prepared. BSA is a 66 kDa protein with an isoelectric point pI = 5.5, and was chosen for this study because it is a well characterized protein, which facilitates theoretical comparisons.

AAO membranes (1 cm in diameter) were functionalized with a polycation in order to promote specific binding of citrate (-COO⁻) gold nanoparticles (containing BSA), by soaking them for 5 hours in a ~5% (weight by weight) solution of polyethylene imimide (PEI) and ultrafiltered deionized water. Polymer excess was removed by immersing the PEI-functionalized AAO membranes into six consecutive baths (10 minutes each) of ultrafiltered deionized water, and dried overnight in a desiccator under vacuum. A gold nanoparticle template containing BSA was deposited on the PEI-functionalized AAO via suction filtration of a 2.5 mL aliquot of the gold nanoparticle solution containing BSA, and vacuum dried for at least 4 hours. The AAO membrane provides a structured planar surface, which is coated with PEI, this facilitates the nanoparticle adhesion in order to form an assembly of a random yet homogenous nanoparticle
layer as shown in Figure 3.4.1(B). Vacuum-filtration upon nanoparticle-BSA incubation, facilitates the assembly of the nanoparticle layer within a small filtration area, limiting sample volumes and concentrating the nanoparticles for subsequent analysis with the WG mode microsphere cavity. Other contents of the solution, such as smaller impurities, excess BSA protein, and smaller nanoparticle pass through the membrane unhindered.

The prepared gold nanoparticle template containing BSA (on AAO) was placed on a glass slide and interrogated with WG mode resonances excited at 633 nm nominal wavelength. The resonant cavity was a silicon dioxide microsphere, 450 ± 10 µm in diameter (SMF-28, Thorlabs), fabricated by melting the tip of a single mode optical fiber, and subsequently coupled to a tapered optical silicon dioxide-microfiber waveguide. The tapering process was done using an oxygen-propane flame. WG modes were excited with a narrow band tunable laser connected (on one end) to an optical taper-fiber waveguide (SMF-28, Thorlabs) eventually connected to a photodetector (on the other end), as described in Figure 3.2.2.

A computer recorded each spectrum and analyzed the spectrum with a polynomial fitting algorithm for Lorentzian-shaped dips in the transmitted intensity, the minimum of which correspond to the resonance wavelength of a WG mode. The resonance wavelength changes were then recorded for different vertical positions of the probing microsphere cavity; i.e. first on the substrate (e.g. nanoparticle layer containing BSA), and then off the substrate (i.e. in air). The WG microsphere is mounted on an XYZ
micromechanical stage, such that different regions of the nanoparticle layer or the capture membrane itself can be repetitively probed for WG resonance displacement associated with nanoparticle binding. The WG setup is mounted on a Nikon TE200 inverted microscope in order to measure proper fiber-cavity coupling.

![Schematic diagram of the set-up employed to evanescently couple WG modes to the Gold nanoparticle (NP) layer. Top: WG mode transmission spectrum for the microsphere in air. Bottom: WG mode spectrum for microsphere in contact with nanoparticles which induce WG mode wavelength shift (continuous line). Adapted from: *Applied Physics Letters* 2011, 99 (7), 073701. Reproduced with permission of the American Institute of Physics copyright 2011.](image)

**Figure 3.4.2.** Schematic diagram of the set-up employed to evanescently couple WG modes to the Gold nanoparticle (NP) layer. Top: WG mode transmission spectrum for the microsphere in air. Bottom: WG mode spectrum for microsphere in contact with nanoparticles which induce WG mode wavelength shift (continuous line). Adapted from: *Applied Physics Letters* 2011, 99 (7), 073701. Reproduced with permission of the American Institute of Physics copyright 2011.

Repetitive WG spectra were recorded in real-time by bringing the WG optical set up in close proximity to the gold nanoparticle layer, then moving it off the substrate in a periodic manner. During this repetitive ‘scan’ of a substrate for induced WG mode wavelength differences, the microsphere cavity remains in fixed mechanical contact with a tapered optical fiber. The WG mode wavelength shifts for two modes, peak 1
and peak 2, were recorded as indicated in Figure 3.4.3. Then, the averaged wavelength displacement of the two WG modes for different probing locations was plotted as a function of BSA concentration in the incubating solution, as shown in Figure 3.4.4. From each measurement, the wavelength shift for a blank substrate consisting of a nanoparticle substrate containing no BSA protein was subtracted. For BSA concentration higher than 10 µM, we observed an additional increase of the shift signal possibly due to BSA multilayer formation and solution aggregation (Figure 3.4.4 inset). Furthermore, we observed slight variation of the shift signal for peak 1 and peak 2 when the gold nanoparticle template was functionalized with BSA at concentrations higher than 10 nM. This finding indicates a possible influence of the WG mode number and the polarization on the wavelength resonance (probing) signal.

Figure 3.4.3. An example of a WG mode spectrum in air (dotted line) and after evanescent coupling to a BSA-nanoparticle layer (continuous line). Adapted from: Applied Physics Letters 2011, 99 (7), 073701. Reproduced with permission of the American Institute of Physics copyright 2011.
The silicon dioxide microcavity probes the nanoparticle layer via its evanescent field. In the case of the 450 ± 10 µm silicon dioxide microcavity, the evanescent field extends about 50 nm at 633 nm wavelength outward from the microsphere surface as explained in the next expression:

\[ L = \kappa^{-1} \cdot (n_{\text{eff},r}^2 - n_{\text{eff},m}^2)^{-1/2} \]  

Eq. 3.4.1

Where \( L_D \) is defined as the penetration depth, \( n_{\text{eff},r} \sim 1.45 \) (at 633 nm) is the effective refractive index of the glass microsphere, \( n_{\text{eff},m} \sim 1 \) is the effective refractive index of the medium surrounding the nanoparticles (approximated as air), and \( \kappa (= 2\pi/\lambda) \) is a constant defining the wave vector.\(^{117}\) The limited extent of \( L_D \) ensures that we are probing only the first layer of gold nanoparticles. Typical Q factors before coupling the microsphere to gold nanoparticle layer are \( \sim 3 \times 10^6 \) at 633 nm; then, after probing the Q-factor minimally decreases to \( \sim 1.5 \times 10^6 \), indicating no drastic decay in microcavity resolution.

The isotherm in Figure 3.4.4 can be explained by a two-component adsorption model, where BSA (small molecule) is adsorbing on a gold nanoparticle surface (large adsorbent).\(^{118}\) Consider a system composed of independent and indistinguishable subsystems; for example, a system of \( M \) adsorbent molecules of nanoparticles with \( N \) adsorbed molecules of protein attached. The thermodynamic equation governing this system is given by:
Figure 3.4.4. WG mode measurements at ~633 nm probing wavelength. The inset shows the BSA adsorption at high solution concentrations compared to the dilute concentration in a log-linear graph. From: *Applied Physics Letters* 2011, 99 (7), 073701. Reproduced with permission of the American Institute of Physics copyright 2011.

\[ dE = TS - pdV + \Gamma dN + \Gamma' dM \]  

Eq. 3.4.2

Where \( E \) is total energy, \( T \) is the temperature, \( S \) is the entropy, \( \Gamma \) and \( \Gamma' \) are the adsorbed and adsorbent chemical potentials respectively. The partition function for each nanoparticle subsystem, consisting of \( m \) equivalent and independent sites for adsorption, is given by:

\[ \xi = (1 + q\Phi)^m \]  

Eq. 3.4.3

Here \( q \) is the concentration, \( \Phi = e^{\mu/kT} \), \( k \) is the Boltzmann constant, and \( \mu \) is the binding energy. The average number \( \bar{N} \) of bound molecules in the two-component system can be estimated from the ensemble of nanoparticles subsystem as follows:
\[ N = M \Phi \left( \frac{\partial \ln \xi}{\partial \Phi} \right)_T \quad \text{or} \quad \bar{s} = \frac{N}{M} = \Phi \left( \frac{\partial \ln \xi}{\partial \Phi} \right)_T \]  

Eq. 3.4.4

Where \( M \) is the total number of available sites, \( \bar{s} \) refers to the average number of adsorbed molecules per nanoparticle subsystem with \( 0 \leq \bar{s} \leq m \). Inserting the partition function for the nanoparticle subsystem into Equation 3.4.4, we obtain:

\[ \bar{s} = \Phi \left( \frac{\partial}{\partial \Phi} \right) [\ln(1 + q\Phi)] = m\Phi \frac{q}{1+q\Phi} = m \frac{q\Phi}{1+q\Phi} \]  

Eq. 3.4.5

Therefore, considering a single nanoparticle interface illuminated by the evanescent field of the WG resonator, Equation 3.4.5 reduces to a classical Langmuir adsorption equation.

**Figure 3.4.5.** WG mode control measurements at two different wavelengths (633 nm and 1064 nm) for gold nanoparticles immobilized on an AAO membrane and gold nanoparticles immobilized on a silicon dioxide slide.
Control experiments were implemented to understand the extent of the nanoparticle enhancement. In order to confirm that the initial plasmonic-photonic presumption was accurate, the WG resonance wavelength shift was measured for bare gold nanoparticles immobilized on an AAO membrane (porous substrate) and gold nanoparticles deposited on a glass slide (non-porous substrate) at 633- and 1064-nm probing wavelengths. For the preparation of the gold nanoparticle template on glass slides; microscope silicon dioxide slides were anodized in a Harrick PDC-32G plasma cleaner, then they were soaked in a ~5% (weight by weight) PEI solution in ultrafiltered deionized water for 30 minutes followed by the addition of as-synthesized gold colloids until the surface of the glass slide was completely covered. After 30 minutes the supernatant was removed and the glass slide was dried in an oven at 100 degrees for 10 minutes. Figure 3.4.5 shows the WG resonance response upon perturbation with both nanoparticle control substrates (with no BSA) at two different wavelengths. For the same gold nanoparticle layer, the wavelength shift averaged over different probing locations is larger at ~633 nm wavelength ($\Delta \lambda = 1.2 \pm 0.1 \times 10^{-4}$ nm) as compared to ~1064 nm wavelength ($\Delta \lambda = 0.8 \pm 0.1 \times 10^{-4}$ nm). This result makes sense since gold nanoparticles probed at 633 nm wavelength should induce a larger perturbation of the WG resonant mode compared to the 1064 nm probing study because of the proximity to the LSP peak of the gold nanoparticles. In addition, particle aggregation influences the WG response upon microcavity interrogation as evidenced by a larger wavelength displacement for the nanoparticle template immobilized on AAO, compared to the ones deposited in glass due to the higher aggregation state. It can be certainly established that gold nanoparticles enhance the detection limit of a WG cavity. Although it would be
tempting to conclude that this enhancement in sensitivity is due to LSP enhancement; theoretical calculations are needed to definitively confirm plasmonic enhancement.

3.5 Microcavity-Particle coupling

One question raised by this strategy is the conservation of the Q-factor upon the cavity-particle coupling. By definition, LSPs arises when a metal nanostructure is excited with electromagnetic waves of a given frequency, which induce an electrical dipole on the nanostructure surface due to the coherent oscillation of delocalized electrons, as illustrated in Figure 2.1.1(A). The excitation of LSPs should consume a considerable portion of the WG mode, which would severely attenuate the Q-factor of the cavity.

Gold nanoparticles were selected for this study due to their plasmonic activity and their chemical stability. The probing wavelength was chosen to be close (~ 100 nm apart) to the LSP wavelength, such that surface plasmons were “mildly” excited. This mild excitation is convenient because the particle polarizability does not expand ad infinitum, allowing us to get the best of two worlds, modal WG confinement and plasmonic enhancement at the nanoparticle surface.

LSPs occur in a restricted regime (usually the nanoscale), which represents a challenge for their direct observation. In order to obtain more detailed understanding of WG mode interactions with the LSP resonances of the gold nanoparticles, numerical calculations based on the generalized multiparticle Mie theory were performed.119-122
This theory provides rigorous semi-analytical solutions of Maxwell's equations for an arbitrary cluster of spheres. It should be stated that the equation derived from the first-order approximation (Equation 3.2.7) cannot be applied in this scenario as it is limited to mode perturbations detected in the form of a WG resonant wavelength shift without accounting for enhancing interactions on the nanoparticle surface.

Experimentally obtained gold refractive index values were used in the simulations.\textsuperscript{123} In order to limit the computational cost of the simulation, a smaller sphere (5\,\mu m) was used as the WG resonator in the theoretical model, the nanoparticle radius was maintained (55\,nm), a 1\,nm microcavity-particle gap was included as part of the calculations, and the probing wavelength for the coupling was 632\,nm. The simulations were performed by a collaborator, Dr. Svetlana Boriskina, a research scientist affiliated with The Massachusetts Institute of Technology. Analogous approaches to study this effect has been done elsewhere.\textsuperscript{121-122, 124}

An illustration describing the system studied in this calculation is provided in Figure 3.5.1. Basically, the resonant mode of a WG microcavity perturbed by a gold nanoparticle is analyzed. The electric field within three different regions labeled as A, B, and C, was represented as a superposition of Mie optical modes, $E_{lm}^{\nu(j)}(\mathbf{r})$,\textsuperscript{119} as shown in Equation 3.5.1, 3.5.2, and 3.5.3. Region A denotes the medium surrounding the system (air in this case); region B and C define the volume of the WG microcavity and nanoparticle respectively. These calculations are performed in a coordinate system with origin $\mathbf{r} = r_{NP}$, similar to the ones reported elsewhere.\textsuperscript{121-122, 124}
**Figure 3.5.1.** A cross-sectional representation of the system used in these calculations.

\[ E^A(\mathbf{r}) = \sum_{\nu,lm} a_{lm}^{\nu} E_{lm}^{(3)}(\mathbf{r}) + b_{lm}^{\nu} E_{lm}^{(1)}(\mathbf{r}) + d_{lm}^{\nu} E_{lm}^{(1)}(\mathbf{r} - \mathbf{r}_{NP}) \]  
Eq. 3.5.1

\[ E^B(\mathbf{r}) = \sum_{\nu,lm} c_{lm}^{\nu} E_{lm}^{(3)}(\mathbf{r}) \]  
Eq. 3.5.2

\[ E^C(\mathbf{r}) = \sum_{\nu,lm} f_{lm}^{\nu} E_{lm}^{(3)}(\mathbf{r} - \mathbf{r}_{NP}) \]  
Eq. 3.5.3

Where \( l \) and \( m \) denote the polar and azimuthal numbers indexing the mode (respectively); \( l = 1, 2, \ldots, \) and \( m = -l, \ldots, l \). \( \nu \) represents the non-polarized Mie modes (TE and TM). \( j \) is used to describes the energy flow; when \( j = 1 \), energy flows in the outward direction from \( r = 0 \), if \( j = 2 \), energy flows inwards, and if \( j = 3 \) there is a superposition of 1 and 2 energy flows. The optical fields are expressed by the following expansion coefficients \( a_{lm}^{\nu}, b_{lm}^{\nu}, c_{lm}^{\nu}, d_{lm}^{\nu}, \) and \( f_{lm}^{\nu} \); representing the illumination field, the field scattered from the WG resonator, the field within the WG resonator, the field scattered from the nanoparticle, and the field within the particle respectively.
These expansion coefficients can be analytically related by imposing continuity of the tangential field components at the WG resonator and nanoparticle surface. It should be remarked that the expansions of $\tilde{d}_{lm}^u$ and $\tilde{f}_{lm}^u$ are performed in a coordinate system with origin $\mathbf{r} = r_{NP}$. Equations 3.5.1, 3.5.2, and 3.5.3 allows the calculation of the spatial field distribution of the local field intensity near the cavity-particle surface. In the frame of the first-order perturbation approximation mentioned earlier,\textsuperscript{91–92} the fractional wavelength shift caused on a spherical microresonator (with permittivity $\varepsilon_r$) by a small protein molecule at a position, $\mathbf{r}_\nu$, influenced by plasmonic enhancement (nanoparticle surface) $\mathbf{r}_{NP} \equiv \mathbf{r}_\nu$ is directly proportional to the field intensity value at the molecule position, $|\mathbf{E}(\mathbf{r}_\nu)|^2$, and inversely proportional to the energy density integrated over the whole mode volume as described in Equation 3.5.4.

$$\left(\frac{\Delta \lambda}{\lambda}\right) = \frac{\varepsilon |\mathbf{E}(\mathbf{r}_\nu)|^2}{\varepsilon_0 \varepsilon_r} \int V |\mathbf{E}(\mathbf{r})|^2 dV$$  \hspace{1cm} Eq.3.5.4

Additionally, the values of the calculated nanoparticle-induced wavelength shifts of the WG mode in the visible and near-IR band are shown in Figure 3.5.2(A). The significant electric field enhancement on the nanoparticle surface can be attributed to the formation of multiple hybrid photonic-plasmonic modes that arises from the coupling of the narrowband WG mode with the plasmon resonance, as exhibited in Figure 3.5.2(B). It has been previously shown that efficient coupling of photonic modes to LSP oscillations can be accomplished in a wide frequency range covering the longer-wavelength slope of the nanoparticle LSP resonance peak.\textsuperscript{121–122} The calculations in
Figure 3.5.2(B) show that coupling to the WG mode results in up to 3 orders of magnitude increase in the electric field intensity on the nanoparticle surface. In the same figure, it can be observed that the peak intensity wavelength of the microsphere-nanoparticle structure is red-shifted from that of the plasmon peak as expected (i.e. plasmon shift due to dissipative losses from dielectric/metal coupling). In addition, as described in Figure 3.5.2(C) strong WG mode coupling to the nanoparticle collective electron oscillations at wavelengths close to the nanoparticle LSP peak increases both radiative and dissipative losses, which translates into a decrease of the cavity Q-factors of hybrid modes in the 520-580 nm wavelength range. This decrease of the cavity structure’s ability to confine energy from the external excitation field is also reflected in the drop of the field intensity observed in Figure 3.5.2(B). This effect is diminished by coupling WG mode to plasmonic nanoparticles outside the LSP region (~ 100 nm apart). Two-dimensional maps describing the spatial distributions of the local field intensity near the surface of the microsphere at the resonance wavelength utilized in this calculations (632 nm) in the presence and absence of the gold nanoparticle are shown in Figure 3.5.2(D-E) respectively.

WG mode resonators store confined energy inside the microcavity material while featuring superior energy accumulation properties and interact with the environment via weak evanescent tails. This translates into a high spectral resolution of the microsphere-based sensors but reduces their sensitivity to ambient changes. In contrast, the electromagnetic hotspot generated on the surface of the plasmonic nanoparticle
is directly accessible to the adsorbed molecules, enhancing the sensitivity of WG resonators without impacting their resolution. Indeed, these calculations utilizing generalized Mie theory\textsuperscript{119,121-122} show that absorption of a single BSA molecule (modeled as a nanosphere with 3.4 nm radius and a $n=1.45$) at the microsphere equator does not produce a detectable shift of the WG mode resonance at $\lambda \sim 580$ nm. However, the same molecule placed in the hot spot created on the microsphere-coupled nanoparticle causes $\Delta \lambda = 1.06 \cdot 10^{-4}$ nm shift of the corresponding hybrid resonance.

\textbf{Figure 3.5.2.} (A) Spectral shifts of the first-radial-order WG mode of a 5$\mu$m-diameter silicon dioxide ($\text{SiO}_2$) microsphere in air caused by the adsorption of a 55 nm-diameter gold nanoparticle (shown in the inset). (B) Intensity enhancement as a function of wavelength on the gold nanoparticle surface in the presence (red dots) and absence (blue line) of the sphere under the illumination by a linearly polarized plane wave. (C) Q-factors of the resonances corresponding to the WG mode in the microsphere.
and hybrid photonic-plasmonic modes in the coupled microsphere-nanoparticle structure. (D-E) Two-dimensional spatial electric field intensity distributions at the wavelengths of a hybrid resonance, \( \lambda_0 = 630.84 \), (D) and the corresponding WG-mode resonance, \( \lambda = 630.819 \) nm, (E) – microsphere surface is shown as a dashed line. From: *Applied Physics Letters* **2011**, *99* (7), 073701. Reproduced with permission of the American Institute of Physics copyright 2011.

The higher sensitivity of the photonic-plasmonic WG mode platform originates from the decrease in mode volume induced by particle coupling. Earlier in this chapter, in section 3.3, the detection limit of a resonator is expressed as a \( Q/V \), and it is stated that in order to achieve higher sensitivity it is necessary to increase \( Q \) while decreasing \( V \). In our developed photonic-plasmonic platform, high-Q microresonators are used; thus we are interested in maintaining the \( Q \)-factor while decreasing \( V \), which is accomplished by the introduction of a hybrid photonic-plasmonic mode in the WG microcavity-nanoparticle structure, greatly improving the BSA detection limit as compared to individual, WG-mode-based\(^{34}\) or nanoparticle based sensors.

### 3.6 Summary

This chapter detailed the combination of silicon dioxide spherical microcavities, sustaining WG resonances, and plasmonic gold nanoparticles as an alternative to enhance the evanescent field of the microresonator. WG modes were excited on silicon dioxide microsphere excited with a red (663 nm) laser and utilized to interrogate a gold nanoparticle layer containing BSA. It was observed that the WG mode wave-
length shifts depend sensitively on the amount of BSA protein bound by the nanoparticles and that the magnitude of the wavelength shift is in proportion to the BSA concentration, which was measured down to pM levels (10 pM). This hybrid photonic-plasmonic approach has enhanced previous WG mode detection limits for BSA, which originally was down to 20 nM.\textsuperscript{34} Control experiments were executed on two different gold nanoparticle layers (aggregated gold nanoparticles on AAO and dispersed gold nanoparticles on glass) without BSA; in this case, the WG modes were excited on a spherical microcavity at 633- and 1064- nm. The larger wavelength shifts observed for the 633 nm probing wavelength for both (aggregated vs. non aggregated) represent a clear indication of the enhancing mechanisms that gold particles provide in the visible range of the electromagnetic spectrum. Additionally, particle aggregation promotes the formation of hotspots on the nanoparticle layer, which is evidenced as larger wavelengths shifts in the control experiments when analyzing the difference in wavelength displacement caused by the gold nanoparticles on AAO vs. the gold nanoparticles on a glass slide for both 633- and 1064- nm probing wavelengths. Generalized Mie scattering theory calculations describing the details of the coupling between the resonant field of the microcavity and plasmonic gold nanoparticles revealed that the evanescent field of the WG mode cavity can efficiently excite surface plasmons without probing at the LSP peak wavelength, in this case the polarizability of the particle does not expand \textit{ad infinitum} which allows for the conservation of light within the microcavity structure. Therefore, no significant degradation in the resolution is observed. These results motivated follow-on detection experiments.
Chapter 4

Ultrasensitive Protein Detection Studies in Real Time***

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4.1 Molecular Detection in Real-Time

Recent progress in material development has catalyzed a boost in the number of label-free detection methodologies with more than 3000 peer-reviewed articles published within the last 20 years (1996 - 2016). Different technologies have been proposed and several mechanisms have been developed for the sensitive detection of specific analytes. These techniques rely on different probing mechanisms including electrical conductance, light scattering and interferometry, surface and localized plasmon resonance, mechanical resonators and optical resonances. A notable advantage of optical techniques over other technologies resides in their ability to explore light-matter interactions
under a broad frequency range of the electromagnetic spectrum, such that specific methodologies can be established based on the optical properties of a given set of analytes.

Optical WG resonators have received special attention due to their high resolution and extraordinary sensitivity to perturbations in close proximity of the resonating cavity.\textsuperscript{34-37, 40, 42, 44, 85, 87, 97, 100, 106, 112-115, 141} However, achieving the goal of single molecule detection of proteins (approximately 10 nm in size) and their interactions in solution require mechanisms for enhancing sensitivity in WG mode biosensing.\textsuperscript{142} Several approaches have been actively investigated, and specific examples for enhancement mechanisms include the use of, self-referenced mode-splitting techniques,\textsuperscript{39, 114} rare-earth dopants,\textsuperscript{110-112, 116} as well as the hybrid photonic-plasmonic WG mode methodology developed as part of this dissertation.\textsuperscript{42} In the latter approach, a WG mode is tuned close to the plasmon resonance of a surface bound gold nanoparticle “antenna” such that a hotspot of high field intensity is created at the nanoparticle site without significant loss of Q-factor through the excitation of LSPs. Molecules binding to the hotspot location perturb the optical field accumulated at the nanoparticle site and dramatically shift the WG mode wavelength. In fact, several orders of magnitude in sensitivity enhancements have been predicted (in the $10^4$ range),\textsuperscript{42, 143} promising label-free single molecule detection in aqueous solution. This would place the study of single proteins interactions within reach. The hybrid photonic-plasmonic sensing concept was first introduced in chapter 3, in which a WG mode biosensor was coupled to a gold nanoparticle layer and different amount of BSA proteins pre-adsorbed to the
A limitation of this approach is the fact that measurements cannot be done directly in solution. Furthermore, real-time analysis is not possible since this method requires extraction, filtration, and drying of the nanoparticle layer before probing with the WG mode biosensor. In addition, proteins are adsorbed randomly within the nanoparticle layer (i.e., outside of plasmonic field enhancements sites), which lowers the sensitivity of the detection. Therefore, there exists a crucial need for novel approaches that enable the detection of proteins in solution at ultralow concentrations via WG modes.

Most of the available knowledge – through conventional characterization techniques – regarding molecular interactions is founded on averaged molecular ensembles. The development of a platform that permit to study analyte interactions in real-time and how they “behave” in solution has been an ongoing research effort for the past decades. The realization of such ultrasensitive methodology would result in novel means to correlate proposed fundamental processes with direct experimental data; allowing scientist to directly probe stochastic, dynamics, and non-equilibrium processes of a few molecules in solution.

At the beginning of this chapter, different alternatives with extraordinary sensitivity have been mentioned; however there still exists a need for the exploration of routes that do not merely allow the detection of the presence of an analyte but that show potential for the further study of the analyte activity. This chapter focuses on the combination of WG mode cavity with plasmonic (gold) nanoparticles in order to achieve
ultra-sensitive molecular detection in a direct and simple approach, providing a novel technology that can be expanded into the study of molecular interactions at ultralow concentrations.

4.2 Real-Time Protein Detection Platforms Utilizing WG Modes

Herein, the previously developed hybrid photonic-plasmonic WG methodology, consisting of WG microcavity and a random gold nanoparticle layer, is integrated into a real-time detection assay of protein molecules at ultralow concentrations through optical trapping at the sites of plasmonic field enhancements. The sensitivity of the improved photonic-plasmonic WG sensor is demonstrated through the detection of BSA protein down to fM solution concentration levels, corresponding to only less than a thousand protein molecules. The motivation behind this work is to provide a novel methodology that can be utilized to detect analytes in a highly sensitive fashion, while showing potential for the execution of fundamental studies.

The plasmonic template utilized in this project was prepared, in essence, according to the protocol described in chapter 3. A similar modification of the Lee and Meisel's method as the one described by Kruszewski and coworkers was employed in this occasion. Briefly, 55 nm (average diameter) gold colloids were prepared by a reduction of hydrogen tetrachloroaurate (10⁻³ M, 100 mL) with sodium citrate (1% weight
by weight, 4 mL). The $10^{-3}$ M hydrogen tetrachloroaurate solution was heated to 100 °C under constant stirring. Then, 4 mL of a 1% trisodium citrate solution was added to the reacting mixture. Two minutes after the addition of trisodium citrate the reacting mixture turned to a dark gray-black color. The reaction was refluxed for 1 hour, finally exhibiting a deep red color.

The effect of the gold nanoparticle coating layer on the adsorption of BSA is explored. Gold-amino terminated nanoparticles were prepared in a similar way to that described by Kunitake et al.\textsuperscript{144} Basically, citrate-terminated gold nanoparticles were functionalized upon preparation with (11-Mercaptoundecyl)-N,N,N-trimethylammonium bromide (10 mg). After addition of the trimethylammonium thiol, the solution changed to a brown-yellowish color, and the reaction was stopped when the solution turned to a dark brown-reddish color. The high affinity between gold and thiol-functionalized molecules like in the case of (11-Mercaptoundecyl)-N,N,N-trimethylammonium bromide is superior to the carboxylate-gold affinity. This preferential affinity between gold and thiol groups allows the formation of monolayers on the gold surface via bonding interactions as described by Allara and coworkers\textsuperscript{72,145} as well as the displacement of lower affinity molecular layers. However, when this coating layer exchange process was tried with gold nanoparticles synthesized according to the procedure described in chapter 3, mild particle aggregation occurred, which could be observed as supernatant aggregates with a golden color. Therefore, the procedure employed in the synthesis of the gold nanoparticles colloids was modified slightly as stated in the previous paragraph. This modification consists of a slight increment of
the amount of sodium citrate to provide a better stabilizing coating layer for the nanoparticle solution. In this case, the sodium citrate aliquot was 4 mL (1% weight by weight) compared to 2 mL (1% weight by weight), which was the amount used in the previous study described in Chapter 3. This particular adaptation did not affect the final diameter of the nanoparticles.

The nanoparticle template on AAO was prepared accordingly as described in chapter 3. AAO membranes of 100 nm diameter were soaked for 5 hours in a 5% (weight by weight) solution of PEI and ultrapure water (18.2 Ohm-cm). Then, they were washed with ultrapure water, by soaking them in six consecutive baths (10 min each), and then they were dried overnight in a desiccator under vacuum. Gold colloids previously prepared were suction filtered through the PEI-modified nanoporous membrane. Then, the gold nanoparticle layer was vacuum dried for at least 4 hours. There was an initial concern regarding the release the amino terminated nanoparticles, as both surfaces should be positively charged. Thus, a control study was done, in which the amino-terminated nanoparticle layer was immersed in ultrapure water overnight, the membrane appeared the same brownish color, which confirmed that the nanoparticles remained on the surface of the nanoporous substrate.

WG mode spectra were excited with a narrowband tunable cavity laser operating at 633 nm nominal wavelength. A silicon dioxide microsphere (radius: 60 – 250 µm) coupled to a tapered optical fiber42 (SMF-28, Thorlabs) was employed as the micro-
cavity probe. The tapering process was done using an oxygen-propane flame. The microspheres were fabricated by melting the tip of a thinned single-mode optical fiber (SMF-28, Thorlabs) with a carbon dioxide laser with a nominal 10 µm wavelength and a 30 W intensity. The implementation of a carbon dioxide laser facilitates a finer control on the final microsphere diameter as it provides a higher degree of flexibility rather than conventional open flame techniques. The melting process occurs due to the absorption band of silicon dioxide at the carbon dioxide laser wavelength (10 µm).146 In this case, the carbon dioxide laser acts as a highly localized heat source, which allows for a finer control of the microsphere size; besides, this implementation permits the refinish of the microsphere by just focusing the laser beam on the resonator surface at a lower intensity, which reduces optical losses due to scattering from the microcavity surface. Then, the microsphere cavity was permanently aligned to the tapered optical fiber with the help of a dual XYZ mechanical stage such that a WG mode is excited along the equator of the microsphere, see Figure 4.3.1. The fiber-coupled microsphere was mounted on mechanical stages in such a way that it can probe via its evanescent field the wetted nanoparticle layer when brought in contact. The WG mode evanescent field has a considerable spatial extend with an approximate evanescent field length of 50 nm in air and 90 nm in water as calculated with Equation 3.4.1, and excites plasmon resonances at several nanoparticle sites in the layer creating a random – yet evenly distributed – array of highly sensitive intensity hotspots.

BSA protein adsorption experiments were performed at several concentrations, ranging from 0.001 pM to 1000 pM. First, a wetted gold nanoparticle layer was brought
into contact with the WG resonant microcavity. Then, 1.5 μL of BSA solution in PBS (pH 7.25) was added to the gold nanoparticle membrane. After addition of the BSA, the solution wicks through capillary action into the membrane and delivers the BSA molecules to the sensor area where a wavelength displacement is recorded as a function of time and analyzed using a custom Labview program that tracks the wavelength shift signal.

4.3 Results

A schematic representation of the instrumentation and employed in this study is shown in Figure 4.3.1 (A-C). (A) First, a wetted nanoparticle layer is coupled to a resonant microspherical cavity; then, a BSA aliquot is added to the nanoparticle layer. (B) The protein adsorption to the nanoparticle layer induces a shift in the resonance wavelength, which is measured and recorded. (C) The microscope helps to position and align the silicon dioxide microspheres with respect to the fiber and the gold nanoparticle substrate. An electron microscope image of gold nanoparticles (approximately 55 nm in diameter) filtered on an aluminum oxide membrane forming a random distribution of nanoparticles is shown in Figure 4.3.1(D).
The stable integration of the microsphere WG mode sensor with the wetted gold nanoparticle layer is critical for achieving ultra-sensitive detection. The silicon dioxide microsphere cavity remained fixed onto a wetted nanoparticle layer. Initial Q-values of the microspheres are in the $10^6$ range. After stable coupling to the wetted nanoparticle layer, the Q-factor drops slightly but remains in the high $10^5$ range. A stable resonance peak at a specific wavelength is recorded before adding protein solution. Then, BSA protein, dissolved in PBS buffer, is added in microliter aliquots. The solution is introduced to the nanoparticle layer by capillary action and immediately probed by the WG mode of the microsphere cavity. The trajectory of the resonance peak...
wavelength $\lambda_0$ is recorded as a function of time, as shown in Figure 4.3.2. As it can be inferred from this figure the wavelength displacement is given by $\Delta \lambda_{eq}$, which is difference between the initial wavelength of resonance ($\lambda_0$) and the final equilibrium wavelength ($\lambda_{eq}$); the term equilibrium is used, in this particular case, to define a state of physical balance; at this point, the wavelength displacement reaches its maximum point, which is also referred as wavelength of saturation, and is indicative of the culmination of detectable BSA adsorption.

![Figure 4.3.2](image)

**Figure 4.3.2.** An example of a BSA adsorption curve, upon BSA addition, the resonance spectra shift drastically and BSA adsorption is observed in real time as an increase in resonance wavelength. From: *Journal of Biophotonics* 2012, 5 (8-9), 629-638. Reproduced with permission of John Wiley and Sons 2012.

The effect of the nanoparticle coating layer on BSA adsorption was explored. For this specific purpose, negatively charged citrate, -COO\textsuperscript{-} terminated, and a positively charged amino, [-N(CH\textsubscript{3})\textsubscript{3}]\textsuperscript{+} terminated, monolayers were chosen to study the effects of surface charge on nonspecific BSA protein adsorption from solution to the gold nanoparticle layer. A new microsphere was utilized for each adsorption experiment,
hence the fractional wavelength displacement \((\Delta \lambda_{eq}/\lambda)\) was normalized to the WG microsphere radius \((R \cdot \Delta \lambda_{eq}/\lambda)\).

**Figure 4.3.3.** Sphere-normalized WG mode shift measured at 633 nm nominal probing wavelength of the gold nanoparticle layer. From: *Journal of Biophotonics* 2012, 5 (8-9), 629-638. Reproduced with permission of John Wiley and Sons 2012.

The microsphere normalized fractional wavelength shift of BSA adsorption as a function of BSA concentration ranging from fM to pM can be observed in Figure 4.3.3. Unexpectedly large resonance wavelength shifts upon particle adsorption are noted, resulting in an unequivocal indication of the high sensitivity accomplished by implemented hybrid photonic-plasmonic WG platform, which is applied to detect BSA protein molecules down to fM concentrations or less than 1000 molecules. Examining Figure 4.3.3 more in detail, a slightly higher protein adsorption to gold nanoparticles with alkylated amino groups is noticed. This observation is not surprising since BSA
is negatively charged in solution at physiological pH, and it is known that BSA forms monolayers on amino-silanized surfaces.\textsuperscript{34} For both cases, the displacement in the probing wavelength becomes larger as the number of BSA protein molecules added to the gold nanoparticle layer increases, and the slope of the sphere-normalize fractional wavelength displacements offsets after concentrations higher than 1 pM.

Additional information can be obtained from these experiments; taking a closer look at Figure 4.3.2, seeing the number of variables collected in this experiment, which is three: concentration, wavelength displacement, and time. The collection of a time stamp allows us to study how fast these proteins absorb onto the gold nanoparticle layer. In the physics realm, there is a parameter termed rate, which is used to account for how quick a given property changes. In this case, the adsorption rate is given by the rate of the sphere-normalized fractional wavelength shift $\frac{R \cdot \Delta \lambda_{eq}}{\lambda \cdot t_{eq}}$, where $t_{eq}$ is the time that it takes for $\Delta \lambda_{eq}$ to occur when BSA is added to the gold nanoparticle layer.

For this adsorption-kinetic comparison, common points were extracted from Figure 4.3.3. A clearer distinction can be made between the negatively charged citrate, -COO\textsuperscript{-} terminated, and a positively charged amino, [-N(CH\textsubscript{3})\textsubscript{3}]\textsuperscript{+} terminated, as shown in Figure 4.3.4. The discrepancy in adsorption rates of the BSA protein adsorbed on the citrate gold nanoparticle layer, compared to the BSA proteins adsorbed to the amino gold nanoparticle layer, can only be definitely confirmed by recording the wavelength displacement as a function of time (as shown in Figure 4.3.2). In other words, whereas Figure 4.3.3 implies that a larger number of BSA protein molecules is perturbing the WG photonic-plasmonic resonant mode, Figure 4.3.4 confirm that observation and
indicates that the adsorption of that larger number BSA protein occurs faster. For both processes the adsorption rate resembled a first order process, as the logarithm of the concentration relate linearly to the rate sphere-normalized fractional wavelength displacement; small deviations from this linear trajectory can be attributed to diffusion difference of the protein molecules wicking through capillary action.

The unprecedented sensitivity of this WG cavity-particle methodology cannot be explained by mere random BSA protein adsorption. In the next section of this chapter, this problem is studied in a more fundamental manner in order to test whether the propagation of surface plasmons enhance the adsorption of BSA protein molecules.

**Figure 4.3.4.** Rate plot of the sphere-normalized fractional wavelength displacement signal corresponding to the different adsorption kinetics of BSA measured in real time for citrate and amino-modified gold nanoparticle layer.
4.4 Optical Trapping

This section describes the implementation of computational studies to elucidate the details of BSA protein adsorption to the photonic-plasmonic WG mode sensor. Initially, calculations framed on the generalized Mie scattering theory are implemented to understand how the hybrid WG cavity particle resonant mode is affected by the presence of a scatterer (BSA protein molecule). Then, Monte Carlo (MC) simulations are merged with optical simulations to understand how the presence of the optical field affect the adsorption of BSA at such small scales.

The optical calculations employed in this study were performed by a collaborator, Dr. Svetlana Boriskina; currently, a research scientist affiliated with The Massachusetts Institute of Technology. In this case, far and near-field spectra of hybrid plasmonic-photonic structures were calculated in the frame of the generalized multi-particle Mie theory, which provides an exact analytical solution of Maxwell’s equations for an arbitrary configuration of L spherical scatters (nanoparticles and/or microspheres)\textsuperscript{147-149}. Similarly to Equations 3.5.1, 3.5.2, and 3.5.3 the total electromagnetic field scattered by the hybrid structure, $E_{SC}$, can be constructed as a superposition of partial fields scattered by each scatterer:

$$E_{SC} = \sum_{(\nu)} \sum_{(lm)} (a_{lm}^\nu N_{lm} + b_{lm}^\nu M_{lm}), l = 1, \ldots L$$

Eq. 4.4.1
Figure 4.4.1. (A) Schematics and two-dimensional spatial field maps of (A) the gold nanoparticle cluster (trimer) excited with external illumination, and (B) a hybrid microsphere-nanoparticle (gold) cluster sensor under the plane wave illumination at 634.9 nm. From: Journal of Biophotonics 2012, 5 (8-9), 629-638. Reproduced with permission of John Wiley and Sons 2012.

Where $N_{mn}$ and $M_{mn}$ are spherical vector wave functions representing the energy flow from the origin in the coordinate system, $l$ and $m$ denote the polar and azimuthal numbers indexing the mode; $l = 1, 2, ..., m = -l, -l + 1, ..., L$. $v$ represents the transverse Mie modes (TE and TM); whereas $a_{lmn}^{\prime}$ and $b_{lmn}^{\prime}$ are the scattering coefficients for the illumination field and the light scattered from the resonator respectively. A matrix equation for the Lorenz-Mie multipole scattering coefficients $(a_{nm}^{l}, b_{nm}^{l})$ was obtained by imposing the continuity conditions for the tangential components of the electric and magnetic fields on the surfaces of nanoparticles and the microsphere, and by truncating the infinite series expansions to a maximum multipolar order $N$. The far field extinction spectra of a hybrid sensor were calculated under plane-wave illumination incident normally on the top on the microsphere. Experimentally measured
gold refractive index values from Johnson and Christy\textsuperscript{123} were used in these simulations. The gold nanoparticle clusters morphology (55 nm nanoparticle diameters, 4 nm minimum interparticle gap) reproduced the experimental values determined by the SEM image as shown in figure 4.3.1(C).

In these simulations, model hybrid structures composed of a 5 µm diameter silicon dioxide microsphere and finite-size planar nanoparticle clusters of three nanoparticles to simplify the numerical analysis. Our previous work confirmed that such scaled model structures provide a qualitative physical picture of the interactions between the evanescent fields of WG modes and plasmonic modes of nanoparticle arrays.\textsuperscript{42} Figure 4.4.1 shows the result of the calculations for a linear nanoparticle trimer with 4 nm wide inter-nanoparticle gaps; (A) under external light illumination, as shown in the diagram, and (B) coupled to the 5 µm silicon dioxide microsphere through a 1 nm wide sphere-particle gap illuminated with a planar wave as shown in the diagram, resulting into even high-intensity electromagnetic hotspots generated in the gaps of the nanoparticle cluster owing to the excitation and strong near field coupling of localized plasmon modes on gold nanoparticles. The hotspot intensities are boosted by orders of magnitude if the nanoparticle cluster is resonantly excited via the field of the WG mode generated in the microcavity,\textsuperscript{42, 121-122, 150-152} which can be directly observed by comparing Figures 4.4.1(A) and 4.4.1(B).

In Figure 4.4.2, the fractional wavelength shift of the hybrid WG-plasmon mode of the sensor is plotted against the number of adsorbed BSA molecules to different spatial
areas of the nanoparticle cluster. BSA molecules themselves are modeled as spherical nanoparticles of 3.4 nm radius and a refractive index of 1.45. As shown in the inset, first four molecules were assumed to adsorb in the area of the highest field intensity hotspot between the gold nanoparticles (blue dots), followed by the next four molecules (shown as red dots), which formed the second molecular layer in the cluster gaps. As can be appreciated in Figure 4.4.2, the top-layer molecules are immersed in the lower-intensity region of the optical mode field. Clearly, molecules that adsorb in the high-intensity hotspots cause larger wavelength shifts than those forming the second molecular layer, and so forth.

![Figure 4.4.2](image)

**Figure 4.4.2.** Fractional wavelength shifts of the hybridized WG-plasmonic nanoparticle mode of the sensor caused by the adsorption of BSA molecules one-by-one. The inset shows the positions of the adsorbed molecules relative to the hot spots formed in the nanoparticle cluster. From: *Journal of Biophotonics* 2012, 5 (8-9), 629-638. Reproduced with permission of John Wiley and Sons 2012.

Binding at high-field intensity regions can, therefore, explain the large wavelength shifts that were measured in our experiments. The large wavelength shift that is expected for binding of the first molecules at highest field intensities might explain the
offset observed in our measurements in Figure 4.3.3 indicating that we do not yet have a large enough signal-to-noise ratio to resolve these first binding events. Although another explanation to this effect relates to the fact that initially, the BSA protein molecules may not necessary adsorb on every high-field intensity spot, and higher protein concentrations trigger the preferential protein adsorption to additional hotspot sites or high-field intensity regions.

In the end of section 4.3, it is suggested that the force generated by the evanescent field at the LSP site is strong enough to drive protein adsorption, as the unprecedented ultra-high sensitivity cannot be explained under ordinary random adsorption. In order to confirm, more rigorously, whether the calculated field strengths between closely spaced nanoparticles can indeed promote the binding of BSA proteins by optical trapping we need to calculate the total time-averaged acting on a nanoparticle illuminated by the evanescent field of the WG mode. In general, the total time-averaged optical force acting on a molecule or a particle illuminated by incoming light can be obtained from the Maxwell’s stress tensor. However, for the molecules modeled as particles with the radii much smaller than the wavelength of incident light the total time-averaged optical force acting on the molecular particle can be calculated within the Rayleigh (dipole) approximation as the sum of the gradient force and the dissipative force:

\[
\langle F \rangle = -\nabla \langle U \rangle + \langle F_D \rangle = \frac{\hbar n}{c\varepsilon_0} \left( \alpha' \nabla \mathbf{M} + \alpha'' \mathbf{k} \mathbf{M} \right)
\]

Eq. 4.4.2
Here, $\langle U \rangle$ is the optical potential, $n$ is the refractive index of the ambient medium, $I_0$ is the incident field intensity, $M$ is the local electric field intensity enhancement at the molecular particle position, and $\alpha = \alpha' + i\alpha''$ is the isotropic complex nanoparticle polarizability, which for the molecular particle of radius $R$ and permittivity $\varepsilon_p$ embedded in the medium with permittivity $\varepsilon_m$ is calculated as follows:

$$\alpha = 4\pi R^3 \varepsilon_0 \varepsilon_m \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p - 2\varepsilon_m} \quad \text{Eq. 4.4.3}$$

For a BSA molecule with radius of 3.4 nm and refractive index $n_p = 1.45$ embedded in water ($n_s = 1.33$) polarizability $\alpha = 4.546 \times 10^{-37}$ [A²s⁴kg⁻¹].

Following Equation 4.4.2, the dissipative optical force component, which acts in the direction of the incident light propagation, is proportional to the imaginary part of the molecular particle polarizability. Therefore, the effect of this component is typically considered negligible for investigating lateral movement and trapping of small transparent dielectric particles. Under the action of the gradient force, the molecular particle drifts toward the region of higher electric field intensity, where the induced dipole has the lowest potential energy. The probability of finding a sufficiently mobile nanoparticle or a molecule at a spatial position $r$ is\textsuperscript{54}:

$$P(r, U) \propto P_0(r) e^{\left[-U(r)\right]/(k_B T)} \quad \text{Eq. 4.4.4}$$

Where $k_B$ is the Boltzmann constant, $T$ is the temperature, and $P_0(r)$ is the corresponding probability without optical fields. A gradient force strong enough to overcome the
Brownian motion needs to be generated in the plasmonic nanoparticle hotspot area in order to drive preferential optical trapping. For the maximum incident field intensity of the WG mode used in the experiment \( I_0 = 2 \cdot 10^9 \text{ [W/m}^2\text{]} \), the optical potential required for trapping a nanoparticle of 3.4 nm radius and refractive index \( n=1.45 \) in aqueous environment, normalized by \( k_B T \ (T=300 \text{ K}) \),\textsuperscript{153-154} is estimated as \( U[k_BT]=1.1026\times10^{-4} \text{ M.} \) The generalized Mie theory calculations of the field distributions in the various random configurations of microsphere-coupled gold nanoparticle clusters with minimum particle separations of 4 nm show that high-intensity hotspots generated in the clusters have high enough field intensity enhancement (\( M \cong 4-9\times10^4 \)) to provide optical potential strong enough to trap a BSA molecule.

The effects of the nanoparticle diameter and the nanoparticle cluster size on the intensity of the localized hot spots generated in the cluster under resonant excitation by the WG mode field have been studied as illustrated in Figure 4.4.3. The results of this calculation study indicate that (analogously to the case of a single microcavity-coupled nanoparticle\textsuperscript{42}) the hot spot intensity (A) is governed by the degree of spectral overlap between the LSP mode in a cluster (B) and a WG mode in the microsphere. Furthermore, the coupling of various LSP modes within clusters\textsuperscript{155} reduces the hotspot intensity (C), which initially drops rapidly with the increase of the cluster size and then saturates in clusters over 10 nanoparticles in size. Coupling between SP modes of nanoparticles added to the cluster results in broadening and red-shifting of the cluster frequency spectrum (D). Overall, the performed calculations demonstrate that hybrid microsphere-cluster structures with nanoparticle diameters in the range
of 30 – 60 nm provide the optical potential for trapping of small protein molecules at the chosen excitation nominal wavelength (633 nm).

Figure 4.4.3. (A) Field intensity enhancement in a hybrid structure composed of a microsphere coupled to a gold nanoparticle dimer as a function of the nanoparticle diameters and wavelength under plane wave illumination (as shown in the inset). The intensity is evaluated in the nanoparticle dimer gap (detector position shown as a red dot). (B) Intensity enhancement spectra of gold nanoparticle dimers of varying nanoparticle diameters and their spectral overlap with the WG mode of the isolated sphere (shown as dash line). (C) Intensity enhancement in the hybrid sphere-cluster configuration as a function of the cluster size. The inset shows the positions of nanoparticle (gray circles) added to the cluster randomly in the order indicated by their numbers and the position where the field enhancement was evaluated (red dot). (D) Intensity enhancement spectra of gold nanoparticle clusters of varying size (labels indicate the number of nanoparticles in the cluster; nanoparticle diameter is 40 nm) and their
Having confirmed that a WG mode coupled to gold nanoparticle cluster has the ability to trap BSA molecules at the site of high plasmonic field intensities, the distribution of BSA protein binding throughout a random nanoparticle layer is explored using MC simulations. The MC methodology was implemented in order to elucidate if the hybrid WG cavity-particle resonance mode affects the adsorption of BSA to the nanoparticle layer. Protein adsorption is a complex process and influenced by many surface properties such as surface wettability, chemical composition and morphology. Various adsorption models including Langmuir adsorption, virial expansion, and scale particle theory have been used to model proteins adsorption on surfaces. Langmuir adsorption and virial expansion seldom predict the correct behavior due to conformation change of protein upon adsorption to the surface, and interaction with other adsorbed proteins on the surface. Scale particle theory incorporates excluded volume and shape effects, which predicts isotherm to be a function of protein shape. The isotherm broadens due to excluded volume and steepens due to attractive interactions between proteins. However, the scaled particle theory is limited to well-defined geometries and does not incorporate multilayer adsorption. Computational models, such as molecular dynamics, and MC, have advantages compared to analytical models listed above, which solve major issues related to electrostatic and pH effects. However, molecular dynamics simulations are limited to single protein adsorption due to the cost of computational time. Cooperative adsorption of an ensemble of pro-
tein can be studied using MC simulation, which provides a large number of configurations for adsorbed proteins with low computational cost. It should be noted that stochastic approach of the MC simulation could be a disadvantage compared to the deterministic molecular dynamic simulation, which provides dynamical information.

MC simulations were performed with a custom written code developed and executed by Dr. Murat Cetinkaya, currently a research scientist at BASF SE, Germany. The model consists of a two-dimensional square lattice simulation with periodic boundaries, where each lattice on the substrate is as large as a BSA protein (approximately 4 nm radius). MC iterations (i.e. adsorption, desorption, and diffusion on the substrate) were performed according to the Metropolis sampling algorithm. The probability of accepting an MC move is:

\[
P = \min \left(1, e^{\frac{-(U_{\text{new}} - U_{\text{current}})}{k_B T}}\right)
\]

Eq. 4.4.5

where \(T\) is the absolute temperature, \(U_{\text{new}}\) accounts the new potential energy of the system, and \(U_{\text{current}}\) denotes the current potential energy of the system. Reduced units are used for simulation parameters. Under non-biased conditions (i.e. when the optical field effects are not involved), the adsorption energy of a single protein molecule was taken as \(-1.0 \ k_B T\). Field effects are taken into account by biasing the probability of finding a non-occupied lattice and also by modifying the Metropolis acceptance criterion:
\[ P = \min \left[ 1, \left( \frac{f_{\text{new}}}{f_{\text{current}}} \right) \cdot e^{-\frac{(u_{\text{new}}-u_{\text{current}})}{(k_B T)}} \right] \]  

Eq. 4.4.6

Where \( f_{\text{new}} \) and \( f_{\text{current}} \) denote the optical field intensity values at the corresponding locations of the substrate. These intensity values are the results of generalized Mie theory field patterns as shown in Figure 4.4.4(A). The cooperative adsorption of the proteins (i.e. the influence of pre-adsorbed proteins on the adsorption of proteins in solution to adjacent sites) is taken into account by adding an extra energy of \(-0.1 \text{kT}\) for each occupied next neighbor lattice. This value has been assigned after checking that the protein adsorption is moderately affected (i.e. Langmuir type of behavior is no longer observed), but the field effects are not suppressed either. Simulations are run for 50 million MC steps, after which the results are found to converge at low protein concentrations and under field effects. Each simulation is replicated 3 times with a different seed using the random number generator of the computer.

Figure 4.4.4 shows the MC simulations results of BSA protein adsorption on nanoparticles with and without the optical field. Cooperative effect among proteins during the adsorption period is also considered for the MC simulation. Figure 4A shows the field intensity map calculated using the Mie theory, where orange to red regions are hotspots. The field intensity data is chosen as an input for the MC simulation as described earlier in the chapter. Figure 4.4.4(B-C) show the final configuration of the MC simulation for 50 and 500 proteins respectively. These results demonstrate that
even for large number of binding events (i.e. in the given scenario of competing binding sites as well as slight cooperativity) BSA molecules accumulate first at nanoparticle junctions, also referred as high intensity hotspots, which supports the initial premise described in this chapter. That is, the large wavelength shifts observed at such ultralow concentrations are due to optical trapping. Figure 4.4.4(D) shows the coverage of hotspots as a function concentration of proteins; it can be appreciated that when the optical field is switched off, the adsorption of BSA molecules occurs randomly on the nanoparticle surface, where the coverage of hotspots is significantly lowered compared to optical field is on (i.e. optical trapping). A similar effect can be observed in Figure 4.4.5; similarly, the BSA protein coverage of hotspots is simulated as a function of nanoparticle diameter (i.e. 40-, 50- and 55- nm) with the optical trapping.

Figure 4.4.4. (A) The field intensity map for random gold nanoparticle is shown. This map is calculated using the generalized Mie-theory. Representative MC simulations
results for N=50 and N=500 are shown in (B) and (C) respectively. (D) BSA coverage, at the hotspot locations, shows clearly a different trend for the light-field on (blue) and the light-field off (red) MC simulations. From: Journal of Biophotonics 2012, 5 (8-9), 629-638. Reproduced with permission of John Wiley and Sons 2012.

Figure 4.4.5. BSA coverage, at the hotspot locations, as a function of gold nanoparticle diameter. From: Journal of Biophotonics 2012, 5 (8-9), 629-638. Reproduced with permission of John Wiley and Sons 2012.

4.5 Summary

In this chapter, we demonstrated a first hybrid photonic-plasmonic WG mode biosensor that measures protein binding down to fM solution concentration levels (less than 1000 molecules). The unprecedented sensitivity towards detection of proteins is explained by optical trapping of proteins at highly sensitive plasmonic hotspots on a gold nanoparticle layer that is coupled to the WG mode biosensor. This approach indicates a promising route towards achieving single molecule resolution in WG mode
biosensors coupled to engineered or random plasmonic nanoantennas. Sample analysis based on the hybrid photonic-plasmonic approach is rapid since detection is potentially sped up through the use of optical forces that deliver proteins to the sites of high field intensity. However, as indicated by the cluster calculations, the LSP enhancement is governed by the size of the nanoparticle cluster, which motivates the exploration of additional avenues that allow for the experimental detection of the WG mode perturbation by a single plasmonic (i.e. gold) nanoparticle scatterer.
Chapter 5

Single Plasmonic Nanoparticle Detection in a Whispering Gallery Microcavity †

†This chapter is an adaptation from a manuscript in preparation to be submitted for publication.

5.1 Whispering Gallery Mode Resonances – An introduction to Mode Splitting

Previously, in chapters 3 and 4, we demonstrated the integration of WG mode resonators with plasmonic nanoparticles (i.e. gold) for further gains in sensitivity due to the excitation of LSPs on the resonator-particle vicinity. The excitation of LSPs was confirmed through theoretical calculations framed in the generalized Mie scattering theory, which indicated that LSPs can be efficiently excited on 55 nm gold particles with the resonant mode of a WG microcavity (excited at 633 nm nominal wavelength) as illustrated in Figure 3.5.2(B-C). Although these results are encouraging, a finer or more optimal utilization of light-matter interactions in the nanoscale can be accomplished by gaining more control of the WG cavity-particle coupling, or more specifically, by carefully limiting how many particles interact with a given WG resonator. In
this way, an ideal balance between plasmonic nanoparticle hotspots and the number of particles coupled to the WG resonant cavity can be established.

The hybrid photonic-plasmonic WG mode methodology has provided unprecedented and remarkable sensitivity, reducing detection limits from pM concentrations to fM concentrations (less than 1000s molecules). However, there exists a point of diminishing returns wherein the utilization of this approach for the detection of single particles in solution comes at the cost of thermally induced resonance fluctuations, which are difficult to decouple from binding events. This particular challenge could negatively impact the widespread utilization of this methodology for the ultrasensitive detection of single molecules and particles in solution. Therefore, there is a demand for the development and optimization of transducing mechanisms, to overcome these limitations.

Earlier in this dissertation, the high resolution of WG resonators has been detailed. The sensitivity of this methodology is attributed to the evanescent field generated at the surface of a WG microresonator. This evanescent tail enhances light-matter interactions, translating into an extraordinary feedback to perturbations in the proximity of the resonator surface. More in detail, the evanescent field of a WG mode microresonator is highly confined along the resonator surface, extending only a few nanometers away from the surface, and thus may detect perturbations in the proximity of the resonator surface. When a particle or molecule interacts with the resonant mode volume of a WG photonic cavity – either by being in close proximity
to the resonator surface or by binding onto its surface – it induces a net difference in the polarizability of the resonator and its surroundings, which alters the optical properties of the system.\textsuperscript{37, 39, 44, 91} This causes a shift of the resonance frequency, broadening of the linewidth, as well as splitting of the resonance mode into a doublet, depending on the strength of the interaction, the strength of the optical mode, as well as the absorption and scattering properties of the particle at the excitation wavelength of the WG mode.

Chapter 3 illustrates WG modes as a confined resonance system in which the resonant wave propagates in one direction only. However, a perfect azimuthally symmetric microresonator can support two counter-propagating modes with the same wavelength, but opposite propagating directions; one traveling clockwise, and the other one counter-clockwise. “Perfection” is hard to achieve, and deviation from this ideal scenario is accounted by real-life material divergence. High-Q resonators (Q > 10\textsuperscript{7}), on the other hand, are efficient confining enough circulating power within the resonator in order to sustain a counter-propagating resonant mode.\textsuperscript{39-40, 44, 112-113, 160} Scattering centers such an inhomogeneity, geometrical imperfection or particle affecting the mode volume break this degeneracy by coupling to one of the modes (i.e. the counter-clockwise), generating a scattered field that can couple back to the clockwise or counter-clockwise propagating modes; this leads to the splitting of the single resonance mode. The lift of this WG mode degeneracy is termed mode splitting and can be experimentally observed in the transmission spectrum as a doublet featuring two
local minima instead of the traditionally described single Lorentzian propagating resonance as illustrated in Figure 5.1.1.

Figure 5.1.1. (A) Cross-sectional plane view of a doubly degenerated WG resonator exhibiting two propagating modes, clockwise (CW) and counter-clockwise (CCW), as well as the single Lorentzian resonance signal at resonant wavelength ($\lambda_0$) and with linewidth ($\gamma_0$) from the transmission spectrum collected at the photodetector site. (B) Cross-sectional plane view of a doubly degenerated WG resonator affected by a scattering center (i.e. inhomogeneity, geometrical imperfection, particle, etc.), which lifts the original degeneracy and splits the resonance signal into a doublet. The resonance wavelengths and the linewidths of the modes in the doublet are denoted as $\lambda_{1,2}$ and $\gamma_{1,2}$ respectively.

One advantages of using WG mode splitting compared to single resonance propagation resides in the self-reference character inherent to this technique. In single-reso-
nance WG mode, the presence of analytes is confirmed by the magnitude of the wavelength displacement of the Lorentzian-like resonance signal. Different effects such as temperature fluctuations mildly affect the resonator structure and refractive index; thereby inducing a displacement in the resonance wavelength. This is difficult to decouple from the adsorption of analytes at ultralow concentrations; additionally, the analyte position within the resonant cavity mode affects the magnitude of the wavelength displacement. In the case of WG mode splitting, the presence of analytes is detected by differences in the splitting peak position (distance between the two peaks), as well as linewidth broadening of the resonant doublet, and the magnitude of these effects are dependent on the polarizability of the analyte. A more detailed explanation of mode splitting as a function of particle polarizability is provided later in section 5.3 of this chapter.

Mode splitting has proven to be a sensitive technique with ability to detect single virions and particles in a self-heterodyne manner. In this chapter, we demonstrated that the high sensitivity of WG mode-splitting can be utilized to detect plasmonic (i.e. gold) nanoparticles (diameters ranging from 20 to 100 nm) with single particle resolution. This study is pivotal for the understanding, from an experimental viewpoint, of the extent of the WG mode perturbation caused by a plasmonic scatterer (i.e. gold nanoparticle) when the WG mode is excited in close proximity (200 nm difference or less) to the LSP resonance. The results provided in this chapter open a new approach for the optimization of the particle size selection for ultrasensitive particle detection, including single molecule sensing.
5.2 Single Plasmonic Particle Detection – Experimental Methodology

Herein, the ultra-sensitive detection of gold nanoparticles is shown by the difference in propagation of a WG mode splitting resonance (wavelength displacement and linewidth broadening) from silicon dioxide photonic cavities possessing two degenerate modes. The mode-splitting difference of the WG resonance signal is utilized as a tool to quantitatively detect the adsorption of particles to the surface of a WG photonic resonator.

Figure 5.2.1. Schematic representation of the instrumental components utilized in these experiments. In essence, a tapered fiber is used to guide the light from a tunable laser into an optical resonator; a photodetector collects transmitted light at the other end of the fiber, and the transmitted signal is monitored. The inset shows a typical doublet in the transmission spectrum. The resonance wavelengths and the linewidths of the modes in the doublet are denoted as $\lambda_{1,2}$ and $\gamma_{1,2}$ respectively.

A schematic diagram of the WG mode set up utilized in nanoparticle sensing experi-
ments is shown in Figure 5.2.1. Briefly, a Differential Mobility Analyzer (DMA) connected to a nozzle was employed to deposit nanoparticles onto the WG resonator. Nanoparticles were carried out in a dilute colloidal solution using a collision atomizer. A remarkable advantage of using a DMA to deposit particles on the surface of a microresonator is that upon atomizing the colloidal solution into polydisperse droplets, the particles are neutralized to maintain a uniform charge distribution. Subsequently, the DMA separates the particles according to their electrical mobility such that the output slit of the DMA only allows particles within a specific size range to exit, and thereafter they land on the surface of the WG resonator. In order to deliver individual particles, we employed low flow rate (approximately 0.02 cm³/s, measured at the nozzle), as well as low particle solution concentrations. The ability to deposit particles from a gaseous phase on a continuous basis (i) minimizes time constraints as particles do not need to diffuse from solution; (ii) prevents Q-factor losses attributed to surface functionalization of the resonator; and finally (iii) circumvents WG mode degradation due to thermal fluctuations from the solution phase.

On-chip high-Q (Q > 10⁷) microtoroidal silicon dioxide resonators able to sustain WG resonances were fabricated following a photolithography protocol as developed by Armani et al. An advantage of utilizing microtoroid cavities relates to the smaller mode volumes, which translate into higher resolution or Q-factors as compared to microspheres. A schematic diagram of this process is shown in Figure 5.2.2. This method employs (100) prime silicon (Si) wafers prepared with a 2 µm layer of silicon dioxide (SiO₂) grown via wet thermal oxidation. Disk-shaped photoresist pads with
controlled diameters were created through photolithography. The circular disks of photoresist act as an etch mask during immersion in buffered hydrofluoric acid (HF) solution at room temperature. The residual photoresist and organic contamination are removed by washing with acetone. Isotropic and selective removal of silicon was done by exposure of the remaining silicon dioxide disks to xenon difluoride (XeF₂) gas at 3 Torr. As a result, the edges of the silicon dioxide disks were equally undercut, leaving circular silicon posts supporting larger silicon dioxide disks. Then, a processing step was performed to selectively heat and reflow the undercut silicon dioxide disks without affecting the underlying silicon support post. In this step, undercut silicon dioxide disk was surface-normal-irradiated using a carbon dioxide (CO₂) laser (10.6 µm wavelength, at a 30W intensity), which drove the melting of the disk along the periphery while conserving the silicon post intact, this melting process is driven due to the strong temperature dependence of the silicon dioxide optical extinction coefficient near 10.6 µm. As the disk diameter is reduced, the effective cross-section available to absorb laser power is decreased and the shrinkage is observed to terminate when a toroid-like silica structure is formed. Beyond this point, continued laser treatment at the same intensity resulted in no observable change of the structure.

The resonators utilized in this study were 40 µm in major diameter and 20 µm in minor diameter. Figure 5.2.3 shows a photograph of the described setup, consisting of two high-resolution cameras with independent outputs, and two XYZ micromechanical stages to properly align the WG microtoroid cavity with respect to the optical
fiber waveguide. The inset consists of a microscope image of a toroidal WG resonator coupled to a tapered optical fiber with gold nanoparticles illuminated at 660 nm. The nozzle observed in the picture has a diameter of 80 µm to assure that the output air covers an area much larger than the toroid microresonator such that the effect on particle distribution on the microtoroid surface is minimized. WG resonances are guided into the resonator through a tapered optical silicon dioxide fiber (SMF-28). Optical spectra of the WG mode splitting were measured by an optical spectrum analyzer with 0.1 nm resolution.

Figure 5.2.2. Schematic diagram (not scaled) describing a detailed protocol for the fabrication of high-Q (Q > 10⁷) silicon dioxide microtoroids.

For each experiment, a two-dimensional matrix describing the transmitted field as the one shown in Figure 5.2.4 was collected from the photodetector and was subsequently processed using a custom program. In the processing step, the developed analysis tool scans each line of the matrix and fitted the transmission dips using a least squares regression tool in accordance with the double Lorentzian function as defined below:
Figure 5.2.3. Optical image capturing the instrumentation utilized in this study. The inset shows WG modes excited at 660 nm on a microtoroid resonator containing gold nanoparticles.

\[ f(\lambda) = 1 - \frac{l_1 \gamma_1^2 / 4}{(\lambda - \lambda_1)^2 + \gamma_1^2 / 4} - \frac{l_2 \gamma_2^2 / 4}{(\lambda - \lambda_2)^2 + \gamma_2^2 / 4} \]  
Equation 5.2.1

Where \( \lambda_1 \) and \( \lambda_2 \) denote the wavelength of the mode splitting resonance peaks, \( \gamma_1 \) and \( \gamma_2 \) are the corresponding linewidths at FWHM, and \( l_1 \) and \( l_2 \) define the relative depths of the resonance from the transmission spectrum.

Figure 5.2.4. Two-dimensional matrix showing the transmitted field collected at the photodetector site. The horizontal axis denotes the frame number corresponding to the spectral acquisition range, whereas the vertical axis indicates the frame number related to the acquisition time. (B) Example illustrating how the developed data processing program works; in this case, the program was run over the whole spectral range.
range (horizontal frames, 1 to 10000) and for each vertical frame (1 to 2500); the three images show extractions from the initial, middle and final frames (left to right) displaying the fitting (green line) according to Equation 5.2.1 (green line).

Figure 5.2.5. (A) Conceptual illustration of the plasmonic nanoparticle detection experiments. The difference in mode splitting of a microtoroid WG mode cavity is analyzed over time as gold nanoparticles are deposited on the surface of the microcavity. The particle size is varied to understand the effect of the particle diameter in the induced polarizability at the microresonator surface. (B) UV-Vis absorbance spectra of the gold nanoparticles utilized in these experiments. The inset plots the measured LSP as a function of particle diameter. (C) The change in the wavelength separation (i.e., amount of splitting) (left) and the linewidths (right) of the resonance split modes as a function of time. Single particle binding is reflected as discrete sudden jumps in the amount of splitting and linewidth difference.

The described methodology was implemented to explore the plasmonic-photonic coupling between gold nanoparticles and the WG resonance mode-splitting of a microtoroid at 660 nm illumination wavelength. A conceptual diagram of this study is
shown in Figure 5.2.5(A). Highly monodisperse gold particles of 20 ± 2, 30 ± 2, 50 ± 3, and 100 ± 5 nm (purchased from Sigma-Aldrich) were deposited on a microresonator and the changes in mode splitting spectra upon particle adherence were monitored in real-time. The absorbance spectra recorded for gold nanoparticles employed in this study are shown in Figure 5.2.5(B); the inset plots the LSP resonance wavelength as a function of the particle diameter, as well as the LSP-to-WG mode probing wavelength difference. A comparison study using a laser in the near-IR regime (1450 nm) was also performed for 30 and 50 nm gold particles in order to understand how the particle detection is affected when the wavelength of the excitation of the WG mode probe is increased beyond the plasmonic wavelength regime of the particles. For each nanoparticle diameter, the performance of the WG mode resonator is evaluated through the mode-splitting spectrum, collected in the forward direction. As described in Figure 5.2.5(C), the perturbation caused by a gold nanoparticle interacting with the WG mode resonator leads to a discrete jump in the difference of the resonance wavelengths (i.e., the amount of mode splitting) and the linewidths of the split modes in the doublet. The magnitude of the particle-induced perturbation in mode splitting depends on the location of each particle with reference to the field distribution of the resonant WG mode volume and the position of the landing particle with respect to previously deposited particles. Particle binding events were collected until the mode splitting were no longer observable in the transmission spectra.
5.3 Single Plasmonic Particle Detection – Results and Discussion

The ultra-high sensitivity of the high-Q (Q > 10^7) resonant cavities utilized in these experiments allows the resolution of individual particle binding events. When the radius of a particle is considerably smaller than the WG mode probing wavelength, the particle-WG-mode interaction induces a dipole represented by the particle polarizability.\textsuperscript{39, 44, 112-113} For a spherical scatterer in the Rayleigh regime (particle size is smaller than the wavelength), the polarizability volume can be expressed in terms of the dielectric permittivity difference between the particle and the medium for a given particle radius as follows:

\[
\alpha = 4\pi R^3 \left( \varepsilon_p - \varepsilon_m \right) / \left( \varepsilon_p + 2\varepsilon_m \right) \tag{Eq.5.3.1}
\]

By monitoring the change in the peak position and the linewidth broadening of the split resonance modes, the change in the induced polarizability when the resonator is perturbed by a scatterer can be calculated as shown by Özdemir \textit{et al}.\textsuperscript{39-40, 44, 113} This calculation can be applied to a multi-particle adsorption case. In this instance, an optical microresonator sustaining two standing wave modes (one clockwise, and the other one counter-clockwise) is perturbed by several particles. After breaking this degeneracy, the resonance mode is split into a doublet with a lower (*) and higher (***) resonant wavelengths (\(\lambda_1^*\) and \(\lambda_1^{**}\)), the linewidths of the doublet are designated by (\(\gamma_1^*\) and \(\gamma_1^{**}\)). The spectral distance, \(\delta\), is given by the coupling coefficient, \(2d\), and
linewidth broadening, \( \Gamma \), is accounted by the damping rate, \( 2C \), of these counter-propagating WG modes:

\[
2d = - \frac{\alpha f^2(r) \omega}{v} \tag{Eq. 5.3.2}
\]

\[
2C = \frac{\alpha^2 f^2(r) \omega^4}{3\pi(c/\sqrt{\varepsilon_m})^2v} \tag{Eq. 5.3.3}
\]

Here, \( c \) is the speed of light, \( \omega \) denotes the angular frequency, which can be expressed as \( \omega = \frac{2\pi c}{\lambda} \), and \( f(r) \) corresponds to a scalar quantity designating the normalized mode intensity distribution at a particle position \( r \). The perturbation on the pre-existing WG mode splitting, in terms of wavelength displacement and linewidth broadening, upon the adsorption of a scatterer in a medium with a dielectric permittivity \( \varepsilon_m \) (i.e. air, \( \varepsilon_m = 1 \)) can be calculated with respect to the resonant wavelength as follows:

\[
\Delta \lambda_1^* = |\lambda_1^* - \lambda_0| = 2d^*, \Delta \lambda_1^{**} = |\lambda_1^{**} - \lambda_0| = 2d^{**} \tag{Eq. 3.5.4}
\]

\[
\Delta \gamma_1^* = |\gamma_1^* - \gamma_0| = 2C^*, \Delta \gamma_1^{**} = |\gamma_1^{**} - \gamma_0| = 2C^{**} \tag{Eq. 3.5.5}
\]

Defining \( 2d \) and \( 2C \) as:
Thus, the wavelength and linewidth perturbations upon particle adherence can be calculated from the experimental values as follows:

\[ 2g = \delta = |\lambda_1 - \lambda_1^*|, \quad 2C = \Gamma = |\gamma_1 - \gamma_1^*| \quad \text{Eq. 5.3.7} \]

Where \( \delta \) and \( \Gamma \) can be obtained from the peak-to-peak distance and linewidth of the split mode respectively. If Equation 3.5.2 is expressed in terms of \( f(r) \) and substituted in Equation 5.3.3, the following expression for the polarizability can be obtained:

\[ \alpha = -\frac{\langle f / \delta \rangle}{\left( \frac{8\pi^2}{3\lambda^3} \right)} \quad \text{Eq. 5.3.8} \]

Based on the polarizability value from Equation 5.3.8, the radius of a spherical scatterer can be calculated by combining Equation 5.3.1 and Equation 5.3.8; after solving for \( R \), following expression is obtained:

\[ 4\pi R^3 \frac{(\varepsilon_p - \varepsilon_m)}{(\varepsilon_p + 2\varepsilon_m)} = -\frac{\langle f \rangle / \delta}{\left( \frac{8\pi^2}{3\lambda^3} \right)}; \quad R = \frac{3}{4\pi} \sqrt{\frac{\langle f \rangle / \delta (\varepsilon_p + 2\varepsilon_m)}{4\pi \left( \frac{8\pi^2}{3\lambda^3} \right) (\varepsilon_p - \varepsilon_m)}} \quad \text{Eq. 5.3.9} \]

For a given change in polarizability, we calculated the detected particle diameter, \( d_\text{d} \) (\( d_\text{d} = 2R \)), and subsequently the number of detected particles on the resonator surface.
by normalizing $\phi_d$ by the nominal diameter of the particle $\phi_N$. Experimentally measured permittivity values were used for particle polarizability calculation.\textsuperscript{123} Although $\Gamma$ and $\delta$ do depend on the interaction of the particle with the WG mode, it should be noted that the ratio of $\Gamma/\delta$ is independent of the particle position on the WG mode resonator,\textsuperscript{39-40, 44} which provides a self-referenced and heterodyned signal, denoting a tremendous advantage as opposed to other sensing schemes relying on single resonance spectral shift, which in turn is extremely dependent on particle position.

In chapters 3 and 4, numerical simulations framed on the generalized multiparticle Mie scattering theory have been implemented in order to understand the details of the WG mode interaction with the LSP resonance of gold nanoparticles.\textsuperscript{42-43} As supported in previous simulations, the polarizability of a plasmonic particle exponentially grow at the LSP wavelength; in this regime, the $\epsilon_p$ for metals is typically negative while $\epsilon_m$ is positive. Hence, when the particle LSP resonance overlaps with the probing wavelength, $|\epsilon_p|$ approximates $2\epsilon_m$, thus the denominator in Equation 5.3.1 approaches to zero, consequently expanding the particle polarizability to infinity. In such cases, the drastic scattering losses in the resonance mode of the WG mode cavity reduces the Q factor by several orders of magnitude,\textsuperscript{42} depending on the number of particles interacting with the WG mode. On the other hand, preceding theoretical studies have also shown that the hybrid photonic-plasmonic modes that emerge as a result of the coupling of the WG mode with the LSP of gold nanoparticles provides a significant electric field enhancement on the particle surface, which can be efficiently excited at the probing wavelength regime (600 nm range) without significant losses.
in Q factor.\textsuperscript{42} On average, the polarizability of a plasmonic particle increases as the particle LSP approaches the probing wavelength.\textsuperscript{43} For the purpose of this study, the diameter of the particle was carefully selected such that the probing wavelength is not close to the LSP of the particle. Ideally, it would be preferable to select one particle diameter, couple that particle to a microresonator, excite WG modes on the resonant microcavity at different excitation wavelengths and calculate the induced polarizability of the particle. However, this approach would require several narrowband tunable lasers and wavelength-specific optical components (e.g., waveguides, prisms). We circumvent this problem by maintaining the excitation wavelength and using particles of different sizes, which have different LSP wavelengths. Hence, we can send individual gold particles of a given size to the surface of a WG mode resonator, analyze the induced mode splitting difference after being perturbed by a gold particle, quantify the induced polarizability volume as a function of particle size, and finally observe how the LSP and WG mode excitation wavelength difference affect the mode splitting of the WG mode cavity.

For each particle size, only the binding or detection events observed as discrete steps or jumps above the background noise were analyzed. In order to assess the information provided by the calculated particle polarizability volume, it is necessary to determine the detected diameter of the particle, which leads to the number of detected particles at each discrete event. Figure 5.3.1(A) summarizes the induced polarizability fluctuation events for 30 nm gold particles binding the surface of the WG
mode microresonator probed with a 660 nm laser. In order to confirm the reproducibility of our methodology, these measurements were repeated on different microtoroid resonators (i.e., T1 and T2) with 20-, 50-, and 100-nm gold particles. Control experiments were done with 30 and 50 nm gold particles at a probing wavelength of 1450 nm for comparison. By just analyzing the averaged polarizability values as a function of nominal particle diameter plotted in Figure 5.3.1(B), it can be noticed that higher polarizability jumps are observed for particles probed in the infrared regime (1450 nm) compared to particles probed in the visible (660 nm). Within the visible realm, higher polarizability events are measured as the particle LSP approached the probing wavelength with exception of the 100 nm particles. After calculating the average particle diameter detected, which is shown in Figure 5.3.1(C); 20, 30, and 50 nm particles exhibited ascendant trend for the sensing experiment at 660 nm; the greater polarizability counts recorded for 30 and 50 nm gold particles at 1450 nm also translated into a higher average particle diameter detected compared to their homologue case at 660 nm. However, as it is described in Figure 5.3.1(D), there was an inverse relationship between the nominal particle diameter and the average number of particles detected in the sensing experiment performed at 660 nm, which in principle makes sense since as particle LSP gets in proximity to the probe wavelength bigger degradation in Q-factor per binding event is expected due to dissipative losses. In other words, fewer events are enough to degrade the resonance splitting mode. For the experiments performed at 1450 nm the average particle count was larger compared to their correspondent results at 660 nm; nonetheless, there was no significant
difference in terms of average particle count for 30- and 50- nm particles at 1450 nm, as shown in the inset of Figure 5.3.1(D).

![Figure 5.3.1](image)

**Figure 5.3.1.** (A) Polarizability volume distribution for 30 nm gold particles adsorbed on the surface of a microtoroid WG mode resonator illuminated at 660- and 1450- nm respectively. (B) Average polarizability volume, (C) average detected particle diameter, and D average particle count for gold particles (20-, 30-, 50-, and 100- nm) as a function of the particle size for the sensing experiments done at 660- and 1450- nm.

These results show a clear trend for particle sensing in the frame of the hybridized WG photonic-plasmonic coupling. As the gold particle LSP approaches the WG mode excitation wavelength in the visible range (660 nm), more single-particle adsorption events are detected. This in principle makes sense, since the photonic-plasmonic coupling becomes stronger as the LSP wavelength approaches the WG mode probing line. Basically, the closer the LSP is to the wavelength of the WG resonance, the larger the
induced particle polarizability dipole becomes when the particle binds the surface of the photonic cavity, which translates into a larger perturbation at the boundary of the photonic resonator.\textsuperscript{42,43} This was clearly evidenced in the smallest particle sensing experiments. For 20 and 30 nm gold particles, more than one particle needed to be adhered to the surface of the resonator in order to see a reactive difference in $\Gamma$ and $\delta$. A similar effect was observed at 1450 nm interrogation wavelength. For the comparison, two particle sizes in the mid-range of the particle diameter distribution were selected. 30 and 50 nm gold particles were deposited separately on the surface of a toroidal WG mode resonator excited at 1450 nm wavelength. In this case, there is no plasmonic enhancement interaction that can boost the induced polarizability upon particle adsorption. As a consequence, more particles adsorbed in close vicinity are necessary in order to observe a reactive jump in mode splitting, which translates into a higher number of particles detected per $\delta$ and $\Gamma$ discrete steps.

5.4 Summary

Herein, it is demonstrated that the mode splitting in an optical WG resonator quantitatively detects plasmonic particles binding at the surface of a resonator at single particle resolution. Discrete changes in resonance peak position, $\delta$, as well as linewidth, $\Gamma$, were employed to effectively calculate how many particles are present per binding event. Typically, as the particle LSP wavelength becomes closer to the WG resonance probing wavelength, single particle detection events were increasingly observed,
lowering the average number of detected particles per measurement. To the best of our knowledge, this work is the first study analyzing the plasmonic-photonic coupling between WG mode resonators and gold particles using mode splitting in a detailed manner. Gaining control over the number of particles coupled to a resonant microcavity is imperative to engineer novel hybrid photonic-plasmonic WG mode systems with precise high field intensity hotspot distribution across the surface of the photonic microcavity. As shown in chapter 4, the gradient force generated at these hybridize WG photonic-plasmonic hotspots is strong enough to trap small molecules (e.g., proteins) from solution,\textsuperscript{43} which can pave the way to a new generation of hybrid photonic-plasmonic WG mode sensors for applications in single molecule detection.
Chapter 6

Conclusion and Future Work

6.1 Conclusion

The detection of analytes in a direct and ultrasensitive manner has been an ongoing research effort for some time now. The realization of such accomplishment will translate into an enormous contribution to the research field in different boundaries of knowledge such as biology, pharmacology, chemistry, material science, and physics.

Molecular sensing requires the development of versatile platforms that allow the execution of clinical studies as well as their utilization in a more fundamental or research oriented scenario. Looking at sensors as a whole, it can be established that every sensor relies on the utilization of some sort of energy to interrogate a given analyte – let that energy be of an electrical, mechanical or optical nature; the presence of a given analyte is confirmed by an alteration of the sensor behavior as compared to steady conditions. One of the most interesting aspects of sensing platforms relying on light relates to the wide spectrum of energies toprobe a given material, and the different behaviors that the material may exhibit when studied at different light
wavelengths or frequencies. In this dissertation, we focus on providing novel material-synthesis methodologies that can be implemented into existing optical sensing techniques to enhance sensitivities and overcome current limitations.

In chapter 2, we developed a stimuli responsive nanoparticle thin film that undergoes spontaneous active release, which is triggered by the alkalinity of the solution. This substrate is used for SERS detection of a HCC biomarker protein,\textsuperscript{23} as well as two different molecular probes specific for RSV and HBV.\textsuperscript{24} SERS has been one of the most active fields in the optical sensing research field; the transport of analytes from a solution phase to the enhancing plasmonic substrate layer, as well as the intimate contact between these two has represented an ongoing limitation. Nanoparticles in solution overcome transport limitations as they allow a faster mixing with analytes; however, these nanoparticles require the utilization of coating layers to prevent aggregation, which prevent intimate nanoparticle contact. On the other hand, the synthesis of metallic nanostructures on solid surfaces overcomes the intimate contact limitations as they do not require coatings in order to prevent aggregation, but this comes at the cost of longer diffusion times between the analyte and the nanostructured substrate. While most approaches to overcome these limitations have relied on mutually exclusive pathways, our developed stimuli-responsive quasiperiodic nanoparticles thin films provide a novel alternative to simultaneously address those limitations. In this case, transport limitations are bypassed by utilizing a sacrificial thin film for the nanoparticle growth that can be dissolved in alkaline media, which releases the nanoparticles into the solution phase; similarly, direct contact is allowed as no passivation
layer is employed on the synthesis of the nanoparticle layer. Additionally, these substrates can be fabricated in a high throughput manner, permitting the future implementation of barcode sensor technology in SERS platforms. Although these results are encouraging, we were intrigued to pursue a more optimal combination of optical platforms and plasmonic substrates, which would allow for the maximization of light-matter interactions at the nanoscale.

As an attempt to fulfill our interest of maximizing light-matter interactions between optical platforms and plasmonic nanoparticles this work evolved towards confined systems that are able to accumulate electromagnetic waves at high field intensities. Among all the different methodologies WG microcavities are able to sustain light at high mode volumes through the confinement of an optical resonance around the circumference of the resonator cavity, providing extraordinary resolutions. The evanescent field, generated as a consequence of the excitation of WG modes, has the ability to detect analytes in a sensitive fashion (down to nm concentrations). However, the ultrasensitive detection of small analytes such as single proteins, which are considerably smaller than the probing wavelength can be challenging. The displacement of the resonant signal in conventional WG mode methodology is influenced by the amount of material bound to the resonator surface that is interacting with the resonant mode. In addition, the evanescent field decays away from the resonator surface. In chapter 3, gold nanoparticles are combined with WG cavities in order to enhance the WG field, which resulted in a sensitivity increase allowing the detection of analytes (pre-adsorbed to the gold nanoparticles) at low pM concentrations; such enhancement is
achieved through the excitation of surface plasmons, which are heavily confined to the nanoparticle surface. Additionally, a theoretical framework in the frame of generalized Mie scattering calculations is established to confirm that gold LSP can be excited at with the optical field of a WG cavity, without affecting the resolution of the resonator by probing near the LSP wavelength of the gold nanoparticle. These results inspired us to further develop this plasmonic-photonic methodology into a real-time sensing platform for the ultrasensitive detection of analytes.

In chapter 4, the previously developed hybrid photonic-plasmonic WG methodology is advanced in order to detect analytes in an ultrasensitive manner, down to fM concentrations in 1µL aliquots, which translates into less than 1000 molecules. At this low concentration regime, detection is only possible through the preferential trapping of molecules to high field intensity nanoparticle hotspots. Mie scattering calculations were implemented to have a notion of the strength of the optical field at the nanoparticle layer, and in combination with Rayleigh dipole approximation it was confirmed that the optical field generated at the gap of two adjacent nanoparticles is strong enough to drive the trapping of a protein molecule. Additionally, MC simulations comparing protein adsorption on two lattice models, with and without the presence of an optical field, confirmed enhanced adsorption at nanoparticle gap, which correspond to the areas of high field intensity also referred as hotspots. This hybridized photonic plasmonic methodology represents a novel alternative to arrest and detect analytes at ultralow concentrations, enhancing our previously established de-
tection sensitivity limit. However, one consideration is that the plasmonic enhancement attributed to this combination of WG cavities and gold nanoparticles is strongly affected by the number of particles in the cluster. Ideally, this issue could be minimized by carefully controlling the number of particles that interact with the resonator; however, such efforts would require single nanoparticle resolution, which is extremely difficult to achieve via WG modes, relying on single resonances, due to thermal fluctuations and diffusion constraints from particles in solution to the surface of the resonator.

Chapter 5 addresses the aforementioned limitations regarding to single nanoparticle detection by implementing WG mode splitting. This is a self-heterodyned methodology for the detection of analytes, as foreign material are not only detected through the spectral difference of the resonance doublet, but also through the linewidth broadening of the split resonance mode. This approach allowed us to directly study the interaction between gold nanoparticles of varying diameters with an intrinsic WG mode splitting resonance. Single gold nanoparticles were detected, and a connection was established between the mode splitting perturbation and the diameter of the detected particles. Additionally, we found that for bigger particles with a LSP wavelength in close proximity to the WG probing line (approximately 90 nm or less) a stronger perturbation of the split resonance mode occurred, which translated in a lower average number of nanoparticles detected before degrading the mode splitting resonance signal. These results not only confirm the theoretical framework devel-
oped in chapter 3, but also provide a direct experimental understanding of nanoparticle and WG mode coupling, which will contribute to the optimization of particle size selection for a given sensing experiment. The development of this hybrid photonic-plasmonic approach has allowed us to make use of the best of two worlds. Namely, plasmonics which has low-Q but highly confined fields within the nanoparticle surface (small mode volume – stronger interactions); and WG mode resonator which have larger mode volume and higher-Q. The hybrid photonic-plasmonic approach allows the achievement of higher sensitivity without degrading the resolution (Q-factor).

6.2 Future Work

As demonstrated in chapter 5, controlling the number of particles coupled to a resonant microcavity is crucial to develop novel hybrid-plasmonic WG mode platforms with precise high field intensity hotspot distribution across the surface of the photonic cavity. An interesting avenue to be explored is the combination of novel nanoparticle delivery systems capable of triggering a stimuli-driven native metal particle release, like the ones developed in chapter 2, which has the potential to yield an additional enhancement as quenching effects – due to the particle passivation layer – are bypassed.\textsuperscript{23-24} This in return, will push towards the development of novel photonic-plasmonic WG mode systems, with precise nanoparticle-cavity coupling.
One of the most stimulating aspects of research is the intrinsic ability to shed light into different directions. For example, a prevailing limitation of WG mode resonators is the thermal influence on the resonant signal. As inferred in previous chapters, dielectric microcavities are not very efficient dissipating the heat that arises from the optical coupling. In essence, the accumulated field within the cavity creates a heat gradient, affecting locally the internal structure of the resonator. Eventually, this creates noise, signal degradation and impacts the sensitivity and resolution of the mode. The combination of plasmonic particles enhances the WG mode signal while providing a conductive material that can be used to manage the internal heat of the cavity – via the evanescent decay of the LSP – without affecting (severely) the resolution of the WG resonance. The further exploration of this effect in combination with our developed photonic-plasmonic coupling could yield promising returns, especially for technological applications.

In the past, optical microcavity development has been heavily restricted to silicon-derived materials including silica,$^{104}$ silicon,$^{162}$ and silicon nitride.$^{163}$ The chemical functionalization of WG mode cavities have been mainly achieved through silane chemistry. The inclusion of different materials is important in order to optimize optical coupling and minimize optical losses. Although rare-earth dopants (active resonators) have been utilized for this purpose; rare-earth dopants are expensive, and assuring a homogeneous distribution within the silica matrix often represents a challenge. Recent progress in material fabrication has made the production of poly-
mer based WG resonators (with high Q-factor) a reality.\textsuperscript{107-109} Their mechanical tunability, ease for the covalent modification of the surface chemical composition, wide availability of refractive indices, and lower manufacturing costs have made polymers attractive materials for the development of WG mode optical microcavities. Thermo-optical properties of polymers place them in an excellent position for the detection of energetic particles, and the direct monitoring of their interactions with a material.

Ultimately, nature has been doing its work fabricating polymeric structures (i.e. proteins) guided through evolutionary processes. Recently, semi-crystalline structural proteins have been explored as WG resonator materials. These structures are able to sustain high Q-factors (Q approximately $10^5$ - $10^6$).\textsuperscript{164} One notable advantage of these proteinaceous resonators is the ease of fabrication accompanied by the low environmental impact associated with their development without sacrificing optical properties – as compared to analogous polymeric resonators. Further control of molecular structure of protein-based resonators will contribute to the development of energy efficient resonators with enhanced optical properties into a widespread array of applications like optical switching, add-drop filters, as well as flexible opto-electronic platforms.
Bibliography


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Selected Publications