Experimental study on surface plasmon induced optical manipulation and imaging

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Experimental Study On Surface Plasmon Induced Optical Manipulation And Imaging

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SUMMARY

As the global standard of living rise, comes demand for improvement in health care. Coupled with a technological progression towards nanotechnology, the evolution of bio-technology from macro-technology is a natural phenomenon. Fueled by the low cost of micro-fabrication, miniaturization of health care devices, while maintaining high through-put, is the ultimate destination. Ever since the first application of surface plasmon imaging two decades ago, the trains of engineering and scientific advancements in surface plasmon applications have never slowed down. Only recently have surface plasmon been applied in particle manipulation, with the promise of nanometer scale of control. In this thesis, the idea of exploiting the propulsive, immobilizing and imaging capabilities of the surface plasmon phenomenon, ultimately on a single platform, is investigated.

Surface plasmon induced micro-particle propulsion is achievable on thin metallic films, using an angle modulated – Kretschmann prism setup. Conventional SPR systems use either a single silver or single gold layer, whereby the former configuration would experience oxidation while the latter configuration would provide a much lower extent of metal enhanced evanescent wave. However, by making use of silver – gold layer configurations, increment in particle velocities can be attained by increasing the thickness of silver embedded by the gold while maintaining the total thickness, without changing the laser beam intensity. Experimental results point to an average improvement of almost

2X the peak velocity of a 40nm Ag – 10nm Au compared to a 0nm Ag – 50nm Au configuration.

Micro-particle immobilization requires a much reduced sample chamber depth, which in this thesis, uses 33 μ m thick sample wells, only one-third in thickness compared to micro-particle propulsion. Subtle contributing factors from thermal convection as well as optical gradient of the exponentially decaying surface wave, determines the dominant effect of either large scale particle organization or particle propulsion. Under suppressed thermal effects, micro-particles experiencing the gradient pull towards the metal surface remain in their lateral positions. However, due to the balance of the contributing factors, including gravity and optical scattering forces, the axial displacement, albeit minute, from the metal surface varies somewhat periodically with time.

Sensitivity of sample refractive index change of up to 10^{-4} is achieved via surface plasmon resonance imaging. The grayscale technique allows quantization of the effective refractive index sensed by the surface plasmons, by dividing the reflected intensities received across the 256 quantized steps of 0 to 255. By using the same experimental setup and sample substrate, refractive indices of various test samples input into the imaging system can be determined from their reflectance in the ensuing grayscale SPR imaging. SPR imaging works on fabricated SU8 structures are done as well. Change in reflected intensity, when descending dielectric micro-particles enter the probing region of the surface plasmon wave, can be monitored. Grayscale image and cross sectional analytical plots of surface plasmon imaged micro-particles is obtained, too.

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1. Introduction

1.1. Motivation

With the rapid development in the integration of nanotechnologies into bio-technology, the demand for scaled-down and high quality healthcare diagnostic tools is on the rise. Hand-held devices that run on microscopic lab-on-chips consisting of nano-scale features would move diagnostic tools both, physically more accessible to the end-users, as well as lower in the healthcare pyramid, whereby cost could be scaled down proportionally to the ease of fabrication. Being able to monitor specific interactions as well as sense or image singular entities have the advantages of low cost, low volume handling, device miniaturization, high level of sensitivity and high parallelism. Therefore the ultimate goal would be the possible and novel generation of miniaturization and integratable analytical devices, operating entirely on light – **extraction** (sample propulsion and isolation), **manipulation** (sample organization and immobilization), and **inspection** (sample imaging and sensing).

Conventional methods such as fluorescence imaging [1], including its spin-off techniques [2, 3, 4], and interference reflectance microscopy [5, 6] have been used to image biological cells and monitor bio-molecular interactions. However such techniques usually render placing the sample in a foreign medium, or the need to label or tag the sample with a dye so as to monitor its interactions, which could bring about cell-death.

Manipulation of cells is fundamental to the core of biotechnology. However, given their small sizes and large numbers, humans are not able to handle them directly without external aid. Current technological level permits the use of optical tweezers [7 – 11] and their derivatives, such as optical vortex generated optical donuts [12] and self-imaged optical bottle beams [13], to maintain control over the position of the cell under investigation. These methods nonetheless enable singular or small number of sample handling. Other techniques include application of electrical forces [14] to enable micro-scale cell manipulation, or the employment of counter-propagating waves [15, 16] to hold the samples in place. These could either cause likely cell-death through electrocution of the cells or require the implementation of a complex optical setup with highly sensitive controls.

Methods to transport similar colloids and suspensions would include utilizing thermal effects, such as thermophoresis (thermophoretic depletion) and convection [17 – 19]. Such thermophoretic transportation would allow a large number of particulate samples to be handled and congregated at a single time; however, due to the continual convective motion of the ambient fluid, investigation into the particles at the monolayer level cannot be achieved. Since the last decade, there has been steady growth in the employment of optically induced evanescent waves in optical waveguides to propel micro-particles in a desired direction [20 – 23]. Mie particles, placed in a fluidic medium, are driven in an evanescent field when these particles encroach into the region of the optical waveguide. This method uses low power for particle transportation, but, there is no natural phenomenon to enable immobilization mechanism of the micro-particles when using

evanescent propulsion, unless additional techniques are implemented to complement it. This could point to a potentially dimensionally large point-of-care device.

Surface plasmon wave is very sensitive towards changes in the refractive index of probed region. Sensitivity towards changes in sample thickness lies in the region of nanometers while differences in index of refraction from as little as 10^{-5} to 10^{-7} can be detected [24 – 26]. Since all materials have a certain index of refraction, the monitoring of activity can be performed without the need for labeling [27], where in most cases would have rendered the death of the biological samples under test. Growth and movement of cells [28, 29] as well as alteration of cellular characteristics, have been monitored using surface plasmon resonance imaging technique. This technique allows the real-time supervision over any alterations in optical properties, and physical changes in the lateral and axial directions. As inline with biological research, compactness of sensing elements [30 – 32] and high throughput capability is highly desired to enable multiple reactions to take place across a large array of assays [33]. This high parallelism [34, 35] is also offered by SPR imaging and thus would make it a very desirable technique to be used. SPR imaging, based on the principles of SPR sensing and spectroscopy, has an additional plus point over its “parent” techniques. On top of the signal responses receivable via the imaging method [36 – 39], a physical image is derivable and this allows image matching of the cell-under-test to be done with a data bank, to allow a parallel identification route. Because of the ability of surface plasmons to sense differing indices of refraction, 2-D grayscale topographical images can also be created with relation to the curved surface of a sample cell or polystyrene bead [40]. This would give a good sense of the

morphological undulations at the bottom side of the cell or particle. Before we end this segment on imaging, an additional point to note would be the tremendous development brought to optical imaging techniques, such as the high-resolution near-field scanning optical microscopies (NSOMs), by surface plasmons. These techniques which utilize the enhanced optical fields at the tips of the probes [41-43] are not diffraction limited, unlike conventional optical microscopy, since the distance between the scanning probes and sample surface is usually only several nanometers wide, which essentially is near-field. Scanning tunnelling microscopy (STM) uses a reversed method of generating enhanced optical fields. The sample surface is excited optically to produce an enhanced electromagnetic field, which in turn would be detected by a scanning probe, capable of up to 3nm resolution [44]. Such an excitation configuration, which makes use of propagating surface plasmons generated from a thin gold or silver film coated on a right-angled prism [45], would be similar to the ones employed in experiments throughout this thesis work.

Magnitude of surface plasmon polariton penetration depth, in the ballpark region of 100nm [46], is at least one order smaller than the dimension of a typical biological cell. SP induced optical manipulation is thus potentially less damaging to biological cells from both physical and chemical point of view. It is favorable towards both, large scale particle organization [47], as well as specific immobilization on selectively patterned plasmonic landscape [48], as surface plasmon induced micro-particle traps are typically 2 orders smaller than conventional optical tweezers [48]. Spatial patterning of cells and micro-particles is useful for the creation of sample arrays, which can be used for basic cell biology research, drug discovery, and high throughput screening applications. The

coming together of these techniques could allow the possibility of a one-stop **extraction**, **manipulation** and **inspection** device. The uniqueness of this all-in-one system lies in that the working mechanism for each capability derives from the same basis of phenomenon, surface plasmon resonance, which further reinforces the integratability of the various techniques on a single platform.

1.2. Objectives

The main objective of this Masters of Engineering thesis is to combine various techniques under the surface plasmon umbrella such that a miniaturized one-stop healthcare diagnostic device can potentially be viable in the near future. The main objective can be divided into the following sub-objectives:

1. To set up an optical system in order to experimentally maneuver microscopic dielectric particles. The maneuvering has to be done on a controllable basis, so as to vary the speed and direction as and when needed.
2. To enable optical binding of micro-particles such that immobilization of these particles can be achieved. Isolation and trapping of micro-particles can be attained by further patterning the surface for selective and parallel 2-D trapping.
3. To obtain a functional SPR imaging system that can be potentially incorporated along with the above mentioned maneuvering and immobilization techniques. For

feasibility test, initial SPR images are to be obtained on conventional setups, using basic samples such as fluidic media and lithographically fabricated structures.

4. To obtain grayscale morphological images of polystyrene micro-particles using surface plasmon imaging technique.

1.3. Originality of Thesis

A customized optical system is established so as to be able to experimentally maneuver microscopic dielectric particles. A Kretschmann-type prism setup is preferred so that the angle-interrogation method can easily be incorporated mechanically. Simple lenses have been used to focus the laser beam onto the metal/sample plane. The maneuvering has to be done on a controllable basis, so as to vary the speed and direction as and when required. A customized top-down movable microscope, built with basic lenses, microscope objective and a CCD, enables rapid detection of the laser spot impinged on the metal/sample interface. Movement of the sample stage is avoided, unless necessary, so that the experimental surface, which might not be absolutely horizontal, is consistent across different sets of experiments. Particle motion is analyzed and evaluated, after importing the recorded video clip using the CCD, on a personal computer.

To enable optical binding of micro-particles, a specially fabricated sample well of reduced depth is used, in place of the initial 100 μ m well. The optics for the interrogating laser beam has been adjusted to enable beam size reduction, instead of beam focusing. This is to minimize the change in angle incidence of the non-paraxial rays, which comes about from a tightly focused beam. Beam size reduction allows the application of a lower power laser, without compromising on the required intensity. Isolation and trapping of micro-particles can be attained by further patterning the surface for selective and parallel 2-D trapping. This is done via electron beam lithography (EBL).

Last but not least, a functional SPR imaging system that can be incorporated along with the above mentioned maneuvering and immobilization techniques is tested out using the same Kretschmann configuration as before. However to increase the signal-to-noise ratio of imaging, the beam is expanded and the power used is much reduced. To enable increased sensitivity, a grayscale span of 0 to 255 of an 8-bit CCD is used as the characterization method for the refractive index. A black-and-white CCD is used instead of a colored CCD for that matter. A graph is subsequently plotted for the received reflected intensity (in grayscale) against the scanning angle. The resultant plot acts as a calibration rule to pin-point and read-off from it, any test-sample's index of refraction. Furthermore, with the intention of applying SPR imaging on SP-immobilized particulate samples, preliminary studies on the lateral resolution as well as detection capability of the presence of dielectric micro-particles in the penetrated region of the surface plasmon wave are conducted, and grayscale images of the morphological surfaces of micro-particles are obtained.

1.4. Organization of Thesis

Extraction here would mean to propel and consolidate, while **manipulation** here would mean to organize or isolate, and immobilize. The flow of the thesis will be as such:

Chapter 1 – A brief introduction to the motivation of this thesis, whereby the novel intention of incorporating **extraction**, **manipulation** and **inspection** capabilities on the same platform, based solely on a single phenomenological method, is mentioned.

Chapter 2 – Bulk of this chapter revolves round the theoretical fundamentals of the surface plasmon phenomenon, from the generation of evanescent waves to the electrodynamics of metal, and the excitation of plasmons by the evanescent waves.

Chapter 3 – The forefront of the intended novelty lies in the ability to **extract** the required sample for investigation. There is a need to be able to maneuver the samples under test to a designated location. This chapter deals with the propulsion of micro-particles as well as the enhancement technique employed to increase the speed of maneuvering.

Chapter 4 – The next step would be to arrest the sample to be investigated at a predetermined proximity. This **manipulation** segment can, however, be further improved by utilizing more localized immobilization spots. This would provide stability and

consistency to the interrogation process, thus minimizing uncertainty in the results obtained.

Chapter 5 – The final stage of this novel application would be to generate an image of the sample under **inspection**. The received image would contain physical (morphological) information of the sample, as well as its biological (optical) state.

Chapter 6 – The thesis would round up with a summary section on the work done and also the work to be carried out, in order to make the initial motivation a possibility.

2. Theory of Surface Plasmons

2.1. Total Internal Reflection Phenomenon – Evanescent Wave

From the basics of optics, when a ray of light propagates from a medium of higher index of refraction to another of lower index of refraction, the ray would be deflected further from the normal to the plane of incidence, compared to the angle of incidence of the ray in the higher refractive index medium, Figure 2.1a. Any further increase in the angle of incidence till the critical angle would result in a special case whereby the refracted ray would be 90° to the normal, Figure 2.1b, where the ray would be propagating along the interface of the high and low media. However, if we increase the angle some more, intuitively, we would predict the ray to experience a total internal reflection (TIR), when the Poynting vector of the wave front of the optical ray would be in the direction as shown in Figure 2.1c.

Figure 2.1 Optical paths of a ray under different incident angles.

However, this is not completely correct, as there is a small component of energy that is still propagating into and along the lower index medium. For a start, the point of re-emittance of the reflected ray does not coincide perfectly with the point of oblique incidence of the incident beam. This optical phenomenon is known as the Goos-Hänchen

shift. This shift can be interpreted as due to the penetration of the ray into the lower index material before it is reflected. The Goos-Hänchen shift would be explained for a waveguide configuration, in the following, using Figure 2.2. Assuming a guided mode within the given waveguide, where n_1 and n_3 are the cladding materials, n_2 the refractive index of the core, the propagation constant, β , of the wave packet can be given as

$$\beta = \frac{\omega}{v_p} = kn_2 \sin \theta_2 \quad (2.1)$$

and the effective waveguide index can be defined as

$$N_g = \frac{\beta}{k} = n_2 \sin \theta_2 \quad (2.2)$$

Figure 2.2 Goos Hänchen shift explained using an optical waveguide layout.

If we consider an optical waveguide setup, referring to Figure 2.2, the lateral shift would be

$$kz_s = \frac{\tan \theta_2}{\sqrt{N_g^2 - n_3^2}} \bullet \frac{1}{\left(\frac{N_g^2}{n_3^2} + \frac{N_g^2}{n_2^2} - 1 \right)^q} \quad (2.3)$$

where k is wave-vector, $q = 0$ for the TE mode and $q = 1$ for the TM mode.

Consequently, the penetration depth of the ray into the cladding medium can be given as

$$h_c d_s = \frac{z_{s,c}}{\tan \theta_2} \quad (2.4)$$

To reinforce the above mentioned principle, let us assume in the case of total internal reflection that there is no transmitted wave. However, this assumption is impossible to satisfy the Maxwell boundary condition using only the incident and reflected waves [49].

Considering the reflectivity equations at an interface as follows,

$$r_{\perp} = \frac{\cos \theta_i - (n_{ii}^2 - \sin^2 \theta_i)^{\frac{1}{2}}}{\cos \theta_i + (n_{ii}^2 - \sin^2 \theta_i)^{\frac{1}{2}}} \quad (2.5)$$

$$r_{\parallel} = \frac{n_{ii}^2 \cos \theta_i - (n_{ii}^2 - \sin^2 \theta_i)^{\frac{1}{2}}}{n_{ii}^2 \cos \theta_i + (n_{ii}^2 - \sin^2 \theta_i)^{\frac{1}{2}}} \quad (2.6)$$

Solving the above equations when, $\sin \theta_c = n_{ti}$ when $\theta_i > \theta_c$, $\sin \theta_i > n_{ti}$, and both $r_{\perp}$ and $r_{\parallel}$ become complex. If we evaluate the transmitted and reflected intensities, $I_t = 0$ and $I_r = 1$, respectively, there must not be any resultant energy transfer across the boundary, although there should be a transmitted wave. The electric field profile of the transmitted field can be given as

$$\vec{E}_t = \vec{E}_{0t} e^{\mp \beta y} e^{i(k_x x \sin \theta_i / n_t - \omega t)} \quad (2.7)$$

Ignoring the positive exponential component, the wavefunction actually depicts a wave that decays exponentially as it penetrates the medium of lower refractive index. This penetrative wave is known as an evanescent wave, or surface wave, propagating in the horizontal plane. It shall be this exponentially decaying wave that stimulates the generation of surface plasmons, the focus of the rest of this chapter.

2.2. Metal Optics – Drude Metal Model

The optical properties of metals can easily be described by their complex dielectric constants, $\varepsilon_m(\omega) = \varepsilon'_m(\omega) + i\varepsilon''_m(\omega)$, which is frequency dependent. These complex properties are a result of the free electrons available within a metal, which experience a certain resistance to motion (since metals do not have infinite conductivity) and also the inter-band transitional states present in most metals [50]. Surface plasmons can be excited in several metals such as magnesium, aluminum, sodium, potassium, gold and

silver. Semiconductors [51, 52] can also fulfill this criterion by being extrinsically doped; however, this is beyond the scope of this thesis.

Considering the electron motions in a metal under the impinging optical waves, would be analogous to a damped oscillator with an external driving force. The motion of the electrons can be given by equation (2.8) [53],

$$m \frac{\partial^2 x}{\partial t^2} + m\gamma \frac{\partial x}{\partial t} + m\omega_o^2 = qE_o \cos(\omega t) \quad (2.8)$$

where m is electron mass, x is the displacement from the mean positions, γ is the damping constant of the metal, ω_o is the natural frequency of a harmonic oscillator due to the restoring force for a bound charge, q is the electron charge and $E_o \cos(\omega t)$ is the applied optical field.

Since the electrons are free flowing, the restoring force is negligible and thus equation (2.8) can be approximated to be (2.9),

$$\frac{\partial^2 x}{\partial t^2} + \gamma \frac{\partial x}{\partial t} = \frac{q}{m} E_o e^{-i(\omega t)} \quad (2.9)$$

Equation (2.9) shows that the electron motions or optical properties depend on the applied field's frequency. By solving equation (2.9) to obtain the polarization induced by the optical field, and combining it with the definition of the dielectric constants, the

frequency-dependent dielectric constant of the metal can be given by equation (2.10) [54],

$$\varepsilon_m(\omega) = \varepsilon_m = 1 - \frac{\omega_p^2}{\omega^2 + i\gamma\omega} \quad (2.10)$$

or rewritten as (2.11),

$$\varepsilon_m = \varepsilon_r + i\varepsilon_{im} = 1 - \frac{\omega_p^2}{\omega^2 + \gamma^2} + \frac{i\omega_p^2\gamma}{\omega(\omega^2 + \gamma^2)} \quad (2.11)$$

where ω_p is the plasma frequency of the electrons. It is due to the combined motions of part of the free moving electrons from a background of fixed positive charges.

This expression is known as the Drude Metal model. This Drude model approximation fits the characteristics of gold and silver particularly well, with $\varepsilon_{Au} = -10.98 + i1.476$ and $\varepsilon_{Ag} = -17.81 + i0.676$. With increasing frequency, the imaginary term becomes increases and ε_m tends to an imaginary function. This imaginary characteristic points to the presence of a phase shift between the induced field in the metal and the applied electromagnetic field, as well as the absorptivity of the metal. Further simplifying the expression, for a small damping constant and assuming negligible phase difference,

$$\varepsilon_m(\omega) = 1 - \frac{\omega_p^2}{\omega^2} \quad (2.12)$$

This simplified expression assumes a collisionless sea of electrons. With consideration for biomedical applications, the metal used has to be biologically compatible with cells and also not undergo excessive oxidation, such as alkali metals, which would generate interference due to the thin layer of oxide formed on the exterior. Thus gold is often used, while silver, which oxidizes quite readily in air, has to be maintained under controlled environment, in typical SPR experiments.

Figure 2.3 Reflectance spectrum of silver [55].

However, a simplistic assumption of free-moving electrons does not fully explain the optical properties of metal, especially that of a noble metal. If we are to observe the reflectance behaviour of a noble metal with increasing frequency, in this example silver, we notice that there are 2 major regions of interest; the sharp dip and rise around 4 eV as well as the fall around 9 eV, which is well beyond the visible frequency spectrum of light [55]. It turns out that the explanation for both phenomena revolves around the resonance frequencies of 2 electronic bands, namely d- and s-bands. The roll-off in reflectance at 9 eV corresponds to the single free electron at the s-band (silver has an electronic

configuration of $[\text{Kr}].4d^{10}.5s^1$), pertaining to the free electron model of Drude's metal model. It was discovered that the electrons of the completely filled d-band are responsible for the changes noticed at 4eV, which depicts the transition of the electrons from d-band to the empty states above the Fermi level. This is also the reason for the various colours characteristic of any noble metal. The d-band electrons being tightly bounded bring about a different facet of the optical properties of metals, compared to the highly mobile s-electrons. The characteristics of these bound electrons can be explained basing on the Lorentz model, a non-simplified version of equation (2.8). Therefore it can be understood that the Drude's metal model is derived from that of the Lorentz model, whereby the natural frequency of a harmonic oscillator due to the restoring force for a bound charge ω_0 is not approximated to be zero.

By solving equation (2.8) without assumption of negligible restoration force, we are able to obtain equation (2.13),

$$\varepsilon_r(\omega) = \varepsilon_r = 1 + \frac{\omega_p^2}{\omega_0^2 - \omega^2 - i\gamma\omega} \quad (2.13)$$

This is known as the Lorentz model for metals, where the optically active electrons in a material are treated as classical oscillators. The electron is considered to be bound to the nucleus by a harmonic restoration force.

From equation (2.11), ω_p , known as the free electron plasma frequency, is given by,

$$\omega_p = \left(\frac{Nq_e^2}{m\epsilon_0} \right)^{\frac{1}{2}} \quad (2.14)$$

Where q_e is the electron charge, N is the density of free electrons, m is the effective mass of an electron and ϵ_0 is the electric permeability of vacuum.

Correlating Equation (2.12), dispersion equation of a metal, to Equation (2.14), it can be seen that any value below the plasma frequency indicates that the refractive index is complex and the penetrating electromagnetic wave decays exponentially from the surface. This issue will be discussed more in the next section.

2.3. Surface Plasmon Resonance Phenomenon

When an electromagnetic (EM) wave impinges on the surface of a metallic surface, most part of the energy is reflected off, while a small component is being absorbed. The collective oscillation of the free electrons will be influenced by this EM wave, and at a certain maximum frequency, known as the plasma frequency, these electrons will be vibrating at resonance. Also at this resonance frequency, all of the p-polarized E-field will be absorbed by the metal surface and all of the photons' energy and momentum will be matched to that of the resulting surface plasmon polariton (SPP). The unique characteristic of SPPs at the interface between a metal and dielectric material is a combination of that of an electromagnetic wave and surface charge [54]. The E-field and H-field can be given by

$$\vec{E} = \vec{E}(z)e^{i(k_x x - \omega t)} \quad (2.15)$$

$$\vec{H} = \vec{H}(z)e^{i(k_x x - \omega t)} \quad (2.16)$$

where k_x is the wave vector of light in the x-direction.

SPPs are highly dispersive, where the energy and momentum are not linearly related by the velocity of light, and the field intensity concentrated at the metal/dielectric interface is strongly enhanced. Figure 2.4 (a) depicts the schematic of the EM/surface charge nature of SPPs. Activation of these surface charges require an E-field normal to the interface, thus the need for a TM polarized light. Figure 2.4 (b) reflects the exponentially decaying nature of the field intensity as we go further away from the surface, $|E|_z$, as a result of the polariton excitation. This field intensity in the z-direction is evanescent, or in other words, non-radiative and surface-bound.

Figure 2.4 (a) shows the transverse characteristic of the EM nature of the surface polaritons, and the surface charges on the metal/dielectric interface. Figure 2.4 (b) shows the field enhancement experienced on each side of the metal/dielectric interface.

The $\delta_{\text{dielectric}}$ and δ_{metal} refer to the penetration depths of the evanescent fields in the positive and negative z-direction respectively, $\delta_{\text{dielectric}}$ is given by

$$\delta_{\text{dielectric}} = \left(k_{sp}^2 - \epsilon_d \left(\frac{\omega}{c} \right)^2 \right)^{-\frac{1}{2}} \quad (2.17)$$

where k_{sp} is the wave vector of the SPP, ω is the frequency of incident light, and, ϵ_d refers to the dielectric constant of the dielectric medium. $\delta_{\text{dielectric}}$ will be the order of half a wavelength, which is in the hundreds of nanometers range, when δ_{metal} is dependent on the metal's skin depth, given by

$$\delta_{metal} = \frac{\lambda}{4\pi\sqrt{\frac{\omega_{\gamma}^2}{\omega^2} - 1}} \quad (2.18)$$

SPPs are strongly damped in their propagation direction (x-direction) as a result of intrinsic dissipation and radiative damping, therefore their propagation length, given by L_x , is defined as the distance covered until the field intensity reaches 1/e [56].

$$L_x = \frac{1}{(2k_{ix})} \quad (2.19)$$

L_x is directly related to the imaginary part of the complex SPP wave vector, k_{ix} . L_x will be predominantly dependent on the complex dielectric function of the metal if the radiative damping can be neglected.

The dispersion relation of SPPs on a smooth semi-infinite metallic surface is given by [54]

$$k_x = \frac{\omega}{c} \left(\frac{\epsilon_d \epsilon_m}{\epsilon_d + \epsilon_m} \right)^{\frac{1}{2}} \quad (2.20)$$

where ϵ_d and ϵ_m are the dielectric functions of the dielectric and metal.

Thus from equation (2.20), we see the relation needed for the coupling of SPPs back into radiative photons.

By assuming real ω and ϵ_d , and that $\epsilon_1'' \ll |\epsilon_1'|$, a complex $k_x = k_x' + k_x''$ is obtained,

$$k_x' = \frac{\omega}{c} \left(\frac{\epsilon_d \epsilon_m'}{\epsilon_d + \epsilon_m'} \right)^{\frac{1}{2}} \quad (2.21)$$

$$k_x'' = \frac{\omega}{c} \left(\frac{\epsilon_d \epsilon_m'}{\epsilon_d + \epsilon_m'} \right)^{\frac{3}{2}} \frac{\epsilon_m''}{2(\epsilon_m')^2} \quad (2.22)$$

Figure 2.5 shows the dispersion curve of a surface plasmon mode as well as the momentum mismatched between it and that of light.

Referring to Figure 2.5, we see that the dispersion relation approaches the “light line”, I, at low frequencies and small k_x , however at larger k_x , there is a mismatch and thus in air

under normal conditions, SPPs cannot be transformed into light. The techniques of coupling light into SPP mode are discussed in a later section – Generation of Surface Plasmon. Therefore as will be mentioned in the following section, a dielectric medium of sufficient dielectric constant must be present to ‘tilt’ the “light line” to position II [57].

2.3.1. Surface Plasmon Coupling Methods

As shown in earlier Figure 2.5, there is a mismatch, Δk , between the “light line” and that of the dispersion curve of SP, thus light does not couple in SPP mode in air under normal conditions. There are several methods to enable coupling [54], but most of the emphasis will be on prism coupling as SPR particle manipulation and imaging can be done most easily through this way.

2.3.1.1. Prism Coupling

Prism coupling method is the most versatile of the coupling methods as it can be used for SPR spectroscopy/sensing and SPR imaging. It is also the most conventional arrangement, encompassing the Otto and Kreshmann configurations. It was first shown in 1972 by Barker [58] that an optical beam can be coupled directly to SPPs via prism coupling method, without using gratings or surface roughening. The dispersion curve can also be measured using this particular technique. A typical prism coupling arrangement is shown in Figure 2.6.

Figure 2.6 A schematic depicting the prism-coupling technique.

It was noted by Barker, referring to Figure 2.4 (a), that as the electric field pattern of the evanescent wave and that of the SPPs is similar, it is possible that there will be coupling between these modes. Considering Figure 2.7, we see that the lateral component of the optical beam in the x-direction is the part that will couple to the SPPs.

Figure 2.7 The interface made by the dielectric material and metal. The k -vector that will be coupled to the SPPs corresponds to the component, k_x .

This analytical view reinforces the fact why SPPs can never be excited in air (dielectric medium of refractive index n_o) directly, rendering the need for a dielectric medium with a larger refractive index, n . Thus equation (2.23) shows the condition needed for k_x to equal k_{sp} ,

$$k_x = k_{sp} = k_o n \sin \theta > k_o \quad (2.23)$$

Upon fulfilling the SPR conditions, there will effectively be zero reflected intensity of TM polarized light off the prism/metal interface.

2.3.1.2. Waveguide Coupling

Unlike prism coupling, this excitation method uses an optical waveguide coated with a thin film of metal in direct contact with the waveguide [30, 32]. Figure 2.8 shows the schematic for waveguide coupling.

Figure 2.8 A schematic showing the waveguide coupled method.

An optical waveguide only supports certain propagation modes for a unique wavelength of light, the condition which is governed by equation (2.24),

$$\frac{2\sqrt{\varepsilon_w}\pi}{\lambda} 2d \sin \theta_i - \gamma_1 - \gamma_2 = 2m\pi, \text{ for } m = 0, 1, 2, \dots \quad (2.24)$$

where ε_w is the dielectric constant of the waveguide, λ is the wavelength of light in vacuum, t is the thickness of the waveguide, θ_i is the angle of incidence, γ_1 is the phase shift introduced by the internal reflection at the waveguide-substrate interface and γ_2 is the phase change of the incident light upon reflection at the waveguide-air interface.

To obtain the required angle using a single wavelength light source for an unknown refractive index sample would be very tedious. Therefore, the most common method for waveguide coupling would be to make use of a broadband light source. Having a full spectrum of wavelengths, the individual wavelengths of light will refract off the walls of the optical waveguide at different angles. Figure 2.9 shows the schematic for the different θ_{in} if a broadband light source is used. This in turn indicates a wide range of possible θ_{in} and one of them will fulfill the requirements for θ_{SPR} .

Figure 2.9 The possible ray-traces within an optical wave-guided mean of coupling to SPPs when a broadband light source is used.

2.3.1.3. Grating Coupling

Another method of generating SPPs is to make use of a metal coated grating. Figure 2.10 gives the schematic for grating coupled method [59].

Figure 2.10 A schematic showing the peaks and troughs of a grating coupler. The height of the gratings is critical towards the efficiency of SPP coupling.

The efficiency at which light can be coupled to SPPs is determined by the height of the grating, where at a critical height, h_c , the reflected intensity goes to zero. The wave vector of the diffracted light in the x-direction can be given by,

$$k_x = k_o \sin \theta_i \pm Nk_{\text{grating}} \quad (2.25)$$

where N is an integer and k_{grating} is defined by $\frac{2\pi}{\Lambda}$ where Λ is the period of the grating.

2.3.1.4. Rough Surface Coupling

Rough surfaces are akin to multiple gratings of differing periods, and thus can match all the wave vector components. Desired surface roughness of metal films, unless finely controlled via electron-beam evaporation or chemical vapor deposition methods, would be very hard to attain and be controlled.

2.3.1.5. Localized Surface Plasmon

Localized surface plasmon (LSP) [60, 61] has a slight difference in characteristic compared to the above mentioned excitation methods, is that LSPs are non-propagating. It uses point-source excitation techniques, such as using an immersion lens with high numerical aperture (NA) in a suitable ambient or using a sharp Near Field Optics (NFO) scanning tip or probe. If we consider a large NA immersion objective to be equivalent to a simple lens, when an expanded beam that enters the objective lens overfills the aperture

stop, referring Figure 2.11, the non-paraxial rays will be refracted the most by the curvature of the lens. Such rays would form the higher-orders light, where a narrow range of them could satisfy the $k_0 n \sin \theta$ condition.

Figure 2.11 Schematic on the typical localized SPR excitation technique. Wide field objectives or objectives with high Numerical Aperture, NA, are used, to increase the span of interrogation angle available.

This method of coupling is very straight forward and is analogous to the broadband interrogation method, where the latter uses a fixed angle but a span of wavelengths, while the former employs a fixed wavelength but a span of angles at the same instance.

2.3.2. Surface Plasmon Modulation Techniques

Considering only a glass-prism without the metallic coating on the hypotenuse face, light incident on the prism/air interface at the critical angle (θ_c) or higher will result in total internal reflection (TIR). In order to generate SPPs with a metal coated prism, TM light

of a specific wavelength has to be incident at the prism/metal interface beyond θ_c . This method of modulation is known as angle modulation at a specific wavelength.

Figure 2.12 A schematic showing the angle modulation method.

At the surface plasmon resonance (SPR) angle, θ_{spr} , for a certain sample with dielectric constant ϵ_s , the SPPs will be coupled to photons, and will be seen as pronounced scattering from the sample. This can only be noticed by using Near Field Optical Microscopy. Figure 2.12 shows the schematic for the angle modulation method.

As a result of this coupling, no TM light will be reflected from the metal/prism interface, and a dark region (depicting the sample location) will be obtained, in stark contrast to the lateral surroundings. Usually a scanning mirror is used to deflect the light from the source or a Goniometer stage is used to rotate the prism-stage in minute steps to attain the desired surface plasmon (SP) angle, θ_{sp} .

Another method of SP excitation is called wavelength interrogation [52, 53]. This method is generally easy to execute as only a white light source needs to be collimated towards the glass/metal interface. Figure 2.13 depicts the wavelength scanning method. As white light contains the full spectrum of visible light, at a fixed particular incident angle (beyond θ_c), θ_{in} , each individual color will be refracted at a unique angle towards the glass/metal interface. This relates to an infinite number of minute steps of interrogation angles. One of the visible colors will satisfy the θ_{spr} condition, and thus by placing a tunable color filter before a detector, at the exit of the light source, we would be able to obtain the λ_{sp} required.

Figure 2.13 A schematic showing the wavelength interrogation method.

The last method is intensity modulation [54], whereby the refractive index of the sample is varied, so that a pronounced dip in reflectance will be obtained, while keeping the θ_{in} constant. In all the above mentioned methods, a normalized reflected intensity plot is obtained. Figure 2.14 shows a typical plot from the above interrogation methods.

Figure 2.14 Reflected intensity plot obtained from a typical modulation technique.

2.3.3. Damping Characteristics of Metal and its Complex Dielectric Constant

The internal damping of an electromagnetic wave propagating along a metal surface, also known as internal absorption, is determined by the imaginary term of the complex dielectric constant. Under optical excitation, the electromagnetic field component of the SPPs generates electron-hole pairs at the Fermi level. Upon de-excitation, conservation of energy leads to the release of phonons or atomic mechanical vibrations, and consequently, thermal radiation. According to principle of energy conservation, at the prism/metal interface of the prism-coupling method [65],

$$R + T + A = 1 \quad (2.26)$$

where R is Reflectance, T is Transmittance and A is Absorption. T will always be equal to zero under total internal reflection condition, while $R < 1$, since A is not negligible for metals. This subtle absorption of energy by the metal layer would raise the surface temperature that could be measured by a photoacoustic cell and the sum of it together with the R recorded would total to unity.

2.3.4. Field Enhancement due to Surface Plasmons – Metal Enhanced Evanescent Wave

The Kreshmann prism-coupled configuration for SP excitation can be considered as a multiple layered media. The reflectance and transmittance, in Fresnel reflection and transmission coefficients for parallel polarization, can be given by [65],

$$R_{//} = |r_{//}|^2 \quad (2.27)$$

$$T_{//} = \frac{\text{Re}\left[\frac{k_{zD}}{\varepsilon_D}\right]}{\frac{k_{zP}}{\varepsilon_P}} \quad (2.28)$$

respectively, where $k_{zD} = \left[\left(\frac{2\pi}{\lambda} \right)^2 \varepsilon_D - k_{zP}^2 \right]^{\frac{1}{2}}$ is the z-component of the wave vector in the

dielectric sample layer, ε_D is the sample's dielectric constant, $K_{zP} = \left(\frac{2\pi}{\lambda} \right) [\varepsilon_P \sin^2 \theta]^{\frac{1}{2}}$ is

the z-component of the wave vector in the glass prism and ϵ_P is the prism's dielectric constant.

Taking into consideration that at the prism/metal interface there is already an evanescent wave being generated, a stronger E-field is observed at the metal/sample interface. This can be deduced from the following E-field expression (2.29) at a distance z from the prism due to a smaller $|\epsilon_D|$ compared to $|\epsilon_M|$,

$$\langle E_{xz}^2 \rangle = \frac{\epsilon_P}{\left(\frac{2\pi}{\lambda}\right)^2} |V_{//z}^2|^2, \langle E_{zz}^2 \rangle = \frac{k_{xP}^2}{\left(\frac{2\pi}{\lambda}\right)^2 |\epsilon_j|^2} |U_{//z}|^2 \quad (2.29)$$

where ϵ_j refers to the dielectric constant of the j^{th} level of the layered media.

This strong enhancement also makes the E-field discontinuous at the metal/sample interface, in the z-direction. The evanescent field within the metal film is a convolution of two evanescent fields, where one is generated at the prism/metal interface, the other at the metal/sample interface. The enhanced evanescent field excited via SPPs at the metal/sample interface is not uniform and varies considerably with respect to the distance from the interface.

Figure 2.15 Theoretical plots showing the discrepancy between the angle of maximum intensity gain and the angle of minimum reflectance.

In addition to the evanescently decaying character of the surface wave, the field decay within the metal film is complicated by the absorption nature of the metal and the physically finite thickness of the metal. Figure 2.15 shows the relation between the maximum E-field enhancement by the SPPs and the resultant reflectance dip observed at SPR condition.

Observation of respective maximum enhancement angle (θ_{EF}) and minimum reflectance angle (θ_{SPR}) reveals that they are not the same [65]. The θ_{EF} will always be slightly smaller than θ_{SPR} . This supposed discrepancy is a result of the fact that the reflection loss at total internal reflection is determined not only by the mean square E-field at the metal/sample interface, but also the decay characteristic of the field within the metal layer.

2.3.5. Plasmon Propagation Length and its Influence on Surface Plasmon Resonance Imaging

In conventional microscopy, the resolution limit is governed by the diffraction of the illumination. However, for SPR imaging, this lateral resolution is determined by the propagation length, $L_x = \frac{1}{(2k_{ix})}$ [56, 66] and decay of the plasmons at the interface of the metal and dielectric sample. Under optical excitation, SPPs obtain their horizontal momentum from incident photons according to the relation $k_{sp} = k_x = k_o n \sin \theta$.

These plasmons oscillate back and forth the metal/sample interface until they either decay back into photons, observed as pronounced scattering, or just dissipate into thermal radiation. As a result, SPPs are not able to laterally resolve features shorter than their propagation length. In addition, since the propagation of SPPs is unit-directional, features with edges horizontal to this direction could only be resolved limited by the diffraction of light. Referring to Figure 2.16 [66], the propagation length of SPPs is dependent on the absorptivity, the imaginary part of the dielectric constant, of the metal used at the illuminating wavelength.

Figure 2.16 (A) and (C) show optical micrographs of silver and aluminum islands of dimensions $6\mu\text{m} \times 6\mu\text{m}$, respectively. (B) and (D) show their respective SPR image, where (B) is barely resolved longitudinally.

However, the choice of metal also affects the sensitivity of the SPPs toward any refractive index change of the sample. Figure 2.17 shows the reflectance dips for two different metals, gold (A) and silver (B), emphasizing on the difference in the slope of the resonance curves. These are simulation curves done for a single layer of metal, respectively, of 48nm thickness, at illumination wavelength of 632.8nm. Since the SPR condition (metal's dielectric constant) for the two metals are different, the resonant dips occur at different angles, despite having the same refractive index for the sample.

The stark contrast between the two plots lies in the slope. The resonance curve for gold is much gentler, and full width half maximum (FWHM) much wider, than that for silver. This can be explained in terms of the absorptivity of the metal. Gold has a dielectric constant under red illumination, $-10.98 + i1.476$ while silver is $-17.81 + i0.676$, where the difference in the imaginary parts of the constants results in this differing slope and FWHM.

Figure 2.17 (A) shows the SPR curve for gold compared to silver (B). The full width half maximum (FWHM) of (A) is more than twice the width of (B).

Silver resonance curve, having a steeper slope, means a greater reflected intensity contrast between two points taken off the plot, at two angles of incidences, which refer to a change in the interrogation angle, compared to gold. This translates to SPPs that are generated on silver surfaces have a greater level of morphological sensitivity towards refractive index difference compared to SPPs excited on gold surfaces.

2.3.6. History of Surface Plasmon Induced Optical Manipulation

The isolation and acceleration of freely suspended dielectric micro-particles via radiation pressure was first achieved by A. Askin [10] in 1969. A laser beam is highly focused so as to generate a gradient force across the beam waist, sufficient enough to pull a targeted dielectric particle towards the principle axis. This allows specific trapping and manipulation of a single bead, however, at a high energy cost. A simple calculation reveals that for a conventional 3D optical trap formed by a highly focused laser beam, an average intensity of $1\text{mW}/\mu\text{m}^2$ or $10^9\text{W}/\text{m}^2$ is usually required [48]. This is considering

only a single optical trap. However, with a view to large scale manipulation of micro-particles, the economies of energy consumption is of critical importance. In 1992, S. Kawata and T. Sugiura [67] reported the movement of micro-sized polystyrene latex particles in the evanescent field of a laser beam. From the paper, it can be deduced that a much conservative amount of energy is required; 10^7W/m^2 for an effective area of $100 \mu\text{m}$ diameter. This is the pioneer in large scale optical manipulation of micro-particles.

Sugiura et al studied the peculiar characteristics of induced particle motion on top of a film [68]. E. Almaas [69] and S. Chang [70, 71] also performed several theoretical studies on the optical force on a micro-sphere placed in an evanescent field. It was in 2001 that Y. G. Song et al tried to explain the force of surface plasmon coupled evanescent fields on dielectric particles larger than the wavelength of light used [72]. It is understood that basing on pure optical phenomenon, quite contrary to the case of an evanescent field, such a particulate body would experience a resultant force directed towards the metal surface. From the middle of this decade, several inspirational articles were published on optically induced organization of micro-particles on a surface. V. Garces-Chavez et al experimented with counter-propagating evanescent waves [73] as well as doing an in-depth study on two dimensional large scale organization induced by surface plasmon polariton excitation [47, 73]. Evanescent waves are propulsive in nature, thus in-order to secure any Mie dielectric bead present in the effective penetration depth of the surface wave; two counter propagating waves would be required. Counter propagating evanescent waves result in regions of energy peaks and troughs. S. A.

Tatarkova et al explained in 2002 that such binary nature of energy levels causes the microscopic beads to remain at an equilibrium position with respect to each other [74].

V. Garces-Chavez et al furthered the extended organization capability of an evanescent wave by plasmon-coupling the surface waves. Under appropriate conditions, the micro-particles can optically be bound to the metal surface because of the gradient force generated by the exponentially decaying metal enhanced evanescent wave. Optical binding experienced by polystyrene micro-spheres was already experimentally and theoretically explained by M. M. Burns in 1989 [75]. M. Righini stretched the experimental fore-front for particle isolation by creating a patterned plasmonic landscape capable of trapping micro-beads at selected locations [48]. Basing on literature, it can be seen that the physical phenomenon experienced by a dielectric particle can be switched between that of a propulsive nature and one of trapping, depending on the physical dimensions and conditions of its immediate surroundings.

2.3.7. Applications of Surface Plasmon in Optical Manipulation

2.3.7.1. Extended Organization of Colloidal Micro-particles By Surface Plasmon Polariton Excitation

Controlled aggregation, manipulation and ordering of large arrays of colloidal particles can be achieved by maintaining the balance between the applications of enhanced optical force and optically induced thermal forces, generated by surface plasmon polariton excitation. These particulate bodies can be hexagonally packed at the center of the beam,

as seen in Figure 2.18 (a), propelled outwards from the center of the beam under the influence of thermophoresis, or made to align and optically-bound in an orderly manner, Figure 2.18 (b) [47].

Figure 2.18 Hexagonal self-packing of a large number of micro-particles as a result of thermal-aided surface plasmon induced optical organization, and self-alignment of micro-particles devoid of substantial thermal influence.

2.3.7.2. Surface Plasmon Two-Dimensional Quasi-Tweezers

Figure 2.19 (a) Schematic of 2-D optical trapping system, (b) self-alignment of micro-particles under minimal thermal effects, and (c) selective parallel 2-D optical tweezers immobilization of micro-particles on a plasmonic patterned landscape.

Two-dimensional quasi-tweezers can be achieved by selectively fabricating metallic gold or silver structures of 40nm to 50nm in thickness and micrometers in diameter, depending on the size of micro-particle to be trapped. Surface plasmons generated at such localized metal spots act as an optical trap on the micro-sized particles, Figure 2.19. A subtle balance of thermal convective force and the weak plasmonic trapping force is required to hold the dielectric particulate body within the premises of the metal structure. Such unique trapping mechanism requires non-focused illumination and thus much lower optical power is needed compared to conventional three-dimensional optical tweezers. Such 2-D arrays of metallic traps points to a potential use in lab-on-a-chip devices entirely operated with light, as well as the application of an optical sieve [48].

2.3.8. History of Surface Plasmon Resonance Imaging

Surface plasmon resonance microscopy, or in present terms, imaging, was first introduced by Rothenhausler et al. in 1988 [76] as an optical imaging technique for low contrast thin film samples. This imaging technique requires the prism coupling method of generating surface plasmon, either by the Otto (1968) or Kretschmann (1971) configuration. Subsequent years to present days saw applications in the imaging of cell/substrate contacts of living cells by Giebel et al. (1999) [77] and imaging of microstructured monolayers by Steiner et al. (1999) [78], as a result of the high sensitivity of surface plasmon polaritons (SPP) toward axial variations in film or coating thickness [30]. Applications requiring high parallelism as well as in-situ monitoring, such as label-free monitoring of DNA interactions, protein micro-array detection and protein-

carbohydrate interactions [79 – 82] and visualization of microfluidic mixture concentrations and refractive index of transparent nanoparticle films [83, 84], highlight some of the advantages of surface plasmon resonance imaging technique.

Commercialized systems, for example those from BiaCore AB, which is a leading company providing sensing and imaging equipment, includes the angle variation modulation method of the surface plasmon resonance phenomenon. Other leading companies such as Thermo Electron Corp. provide alternative polariton excitation via wavelength modulation technique.

2.3.9. Applications of Surface Plasmon in Imaging

2.3.9.1. Surface Plasmon Resonance Imaging of Organic Thin Films

One of the first applications of SPR imaging involved the imaging of biomolecular surface structures. SPR imaging is effective in such application due to the surface sensitivity of SPPs. This high sensitivity allows the characterization of self-assembled monolayers (SAM) and biopolymer films on metallic interfaces [85, 86]. As the metallic surface is coated with the SAMs over time, the SPR images will change to reflect the immediate alterations. Also, the probing depth (penetration depth) on the metal/dielectric sample side ranges to hundreds of nanometers, means this probing region can be used a nano-scale ruler. The SPR conditions alter according to the effective refractive index of the dielectric medium, which includes the thickness of the sample.

2.3.9.2. In-situ Monitoring and Imaging of Biomolecular Interactions

With an increasing demand for in-situ monitoring of protein-DNA interactions, SPR imaging offers an ideal avenue over conventional methods, as such the necessity of fluorescent markers is eliminated while allowing real time operation. Figure 2.20 [37] gives a comparison between the much matured fluorescence microscopy compared to SPR imaging, with a focus on the in-situ process capability, parallelism operation, sensitivity and the need for labeling.

Criterion	fluorescence	SPRI
Real time operation	-	+
Number of spots in parallel	++	+
Sensitivity	-	+
Necessity of markers	-	+
Maturity of processes	+	-

Figure 2.20 Comparison between fluorescence and SPR imaging.

As biomolecular interactions take place, the effective refractive index of the probed dielectric medium changes. It is through this subtle change in molecular character and the subsequent change in optical property that the SPR technique is able to differentiate the before- and after-states.

Referring to Figure 2.21, with the need to tag the analytes (so that any successful molecular bonding or interactions can lead to a fluorescent resonance energy transfer, FRET [87]) removed before any successful bonding with the ligands, the complexity of

fabrication of immunoassays can be minimized, as well as not rendering the ambient of the analytes or proteins, foreign and harmful.

Figure 2.21 Shows the different immunoassays required for SPR imaging (a), and fluorescence imaging (b).

3. Surface Plasmon Induced Optical Propulsion of Micro-particles – Two-layer Metal Enhancement

3.1. Overview of Chapter 3

Such two-layer metallic film configuration for enhancing the SP field holds promising applications in colloidal science and biophotonics. This could allow faster particle speed during micro-manipulation, without risking the possibility of a pure silver coating undergoing environmental oxidation. With a larger interaction area and guiding efficiency, the proposed configuration permits the use of lower power lasers, without compromising the competence at all. We have made experimental comparisons of the average particle velocities achievable for various metal layer combinations, allowing better control in surface plasmon particle propulsion/manipulation as well as large-scale colloidal organization.

3.2. Concept of Two-layer Metallic Film Induced Surface Plasmon for Enhanced Optical Propulsion of Micro-particles

From where is let off in Chapter 2, an exponentially decaying wave is induced on the other side of a Kretschmann configuration, from where the impinging excitation beam strikes. Now consider a Mie particle made of dielectric material, such as a polystyrene or silica bead, when placed in a medium of deionised water. The particle would sink towards the bottom of the well that holds the body of water, nearing the metallic surface

of the prism-based Kretschmann configuration. When there is no optical illumination, the particle would be purely under the influence of Brownian motion, whereby the underside of the particle would merely hover slightly above the metallic interface. At the metal/water interface, it is known that a wave propagates along the surface, in the direction of the k -vector of the Transverse-Magnetic (TM) polarized interrogation beam. This wave has an exponentially decaying nature of electric field in the direction perpendicular to the metal surface, which is characteristic of a surface wave, and thus corresponds to a very short propagation length along the metal/water interface. A Mie particle with size much larger than the excitation wavelength, would impede, rather than oscillate in accordance to, the decaying surface wave. This impedance couples with interference of the multi-reflected light between the underside of the particle and the metal surface, vertical absorptive and scattering forces, horizontal surface force, gravity and thermal gradient as a result of the metal's thermally-conductive nature. This represents an intricate relationship between the mentioned possible sources of influence, whereby the forces can either be optically or thermally dominant, depending on the intended application.

For the case of optical propulsion, a dielectric particle 200nm from the metal surface would experience an electric field enhancement of approximately 3X in the horizontal direction, compared to non-metal evanescent wave propulsion. For most applications, SPs are excited on a single gold transduction film to achieve stable test results. Gold, though having excellent chemical stability, does not enhance the evanescent field as well as silver. Potentially, any enhancement to the SP evanescent field should yield a device

capable of trapping microparticles over a significantly larger surface area and propelling them at a faster pace. In this chapter, the experiments were carried out on single metal (gold) as well as bimetallic (gold coated on silver) configurations for comparison. Judging from literature, a straight forward method of enhancing the evanescent field will be to employ a two-layered silver-gold configuration to extend the SPs penetration depth [88]. In this chapter, we study in detail the propulsion forces translated to microparticles from SPs excited on various metallic film configurations. The key advantage of the proposed configuration is that the underlying silver yields better evanescent field enhancement, while the overlying gold ensures that the stability of the sensing surface is not compromised. In addition, this SPR configuration can easily be realized as the two-layered metallic structure can consecutively be deposited with an electron-beam evaporator without breaking vacuum.

3.3. Experimental Setup

The optical phenomenon behind SPR imaging lies in the high sensitivity SPPs have towards surface morphologies. The SPPs cannot differentiate between debris and samples under investigation. Thus the condition of the sensing or imaging surfaces must be free from all organic debris and dust particles before coating any metallic layer on the glass prism surface. In this work, metal is coated on top of a standard microscope glass slide such that these substrates are disposable, while allowing the high refractive index prism to be recycled and yet suffer minimum damage to the hypotenuse face of the prism. In order to maintain SPR condition at the glass slide/metal interface, index matching oil is applied

between the microscope slide and the prism prior to any imaging work. This is to bridge the air gap between the two solid surfaces and still allow optical rays to undergo total internal reflection while propagating inside the lower-indexed glass slide. A media of complex dielectric constant has to form a boundary with a dielectric material in order for SPPs to be excited.

As mentioned in Chapter 2, silver has a better reflectivity response compared to gold or aluminum, however due to practicality issues such as oxidation of the metal surface, most research groups stick to using gold, which also doubles up as an inert material towards biological cells of interest. Ong et al. [88] suggested using a bimetallic layer with gold on top of silver to obtain a middle ground of good reflectivity response as well as protection against atmospheric oxidation of silver, which would alter the SPR resonance curve with increasing thickness of oxide grown. Using an electron-beam (e-beam) evaporator inside a clean room environment, silver is directly deposited onto cleansed glass slides (process mentioned in the previous section). A very thin layer (usually 1~2nm) of chromium can be pre-coated onto the glass surfaces to enhance the bonding with the primary metal (gold/silver layer). However no chromium is used here since the idea of the glass slides is to enable disposability, thus unnecessary steps can be avoided. Following that, a layer of gold is e-beam evaporated onto the silver without breaking vacuum.

3.3.1. Fabrication of Sample Wells

A circular sample well of 10 mm diameter, was engraved onto a double-adhesive Mylar (100 μ m thick) using a direct-write CO₂-laser cutting system (Epilog Legend, model: 24TT), and the finished design was pasted onto the metal-coated disposable glass slides. A 160 μ m thick cover-glass slide was stacked on top to seal the chamber.

3.3.2. Angle Modulated Prism Coupling Setup

The Kretschmann prism-coupling configuration, as depicted in Figure 3.1, was used for all our particle propulsion experiments. The incident beam, a 1064 nm laser (max 5W, CW, Yb-fiber laser, IPG Corporation), was first *p*-polarized and made to pass through a convex lens, before striking a mirror which directed light into a high-index prism (ZF13 glass, $n \sim 1.77$ at 1064nm). With a laser power of ~ 300 mW, an effective beam spot intensity of 3.62W/mm^2 impinged the prism/metal interface. The substrates for our experiments were placed on the hypotenuse surface of the prism and low viscosity microscope immersion oil (Resolve®), with refractive index of 1.515, was applied between the glass slides and the prism to bridge the air gap between them. The imaging system comprised a 20 $\times$ objective lens (NA 0.4), with a Hamamatsu IR CCD camera to monitor the particles movement. A video recording software was used to capture the images continuously. An IR cutoff filter was placed in the optical path of the reflected light to enable clear observation of the particle movement, while an iris was employed to enhance the imaging contrast. To investigate the angle dependency of SPR induced

particle movement, the scanning angle was varied at a step size of 0.25° in the ballpark of the resonance angle.

Figure 3.1 Instrumental setup for observing microparticles propulsion.

3.4. Results Analysis and Theoretical Explanation

3.4.1. Calculation of Surface Plasmon Wave's Penetration Depth

The penetration depth of an exponentially decaying plasmon wave is dependent on the k -vector of the generated surface plasmon as well as the dielectric constant of the dielectric coupling medium. In view of the bimetallic configurations that would be used in the propulsion experiments, an approximate range of probing depth into the dielectric medium is calculated basing on a pure silver layer and pure gold configuration. The

penetration depth of surface plasmon wave into the targeted dielectric medium is given

by $\delta_{\text{dielectric}} = \left(k_{sp}^2 - \epsilon_d \left(\frac{\omega}{c} \right)^2 \right)^{-\frac{1}{2}}$. Therefore $\delta_{\text{dielectric}}^{Au} \approx 280nm$ and $\delta_{\text{dielectric}}^{Ag} \approx 360nm$.

3.4.2. Non-Uniform Propulsive Force Across Focused Beam Spot

Since the incident beam intensity profile is Gaussian in nature, the amplitude of the surface plasmon wave would not be equally distributed across the beam spot. Light scattered by the particles close to the metal surface is observed in Figure 3.2. Referring to the figure, the effective beam spot is approximately 100 μm in diameter, where it consists of a tightly focused region (hence the increased scattering) demarked by the intensely white portion.

Figure 3.2 Light scattered by the particles close to the metal surface. The effective beam spot is approximately 100 μm in diameter, where it consists of a tightly focused region (hence the increased scattering) demarked by the intensely white portion.

This implies unequal propulsion forces acting on particles which are scattered over a region and therefore, several particle-velocities were averaged to obtain the mean particle speed. For every metallic film combination, particle propulsions on 5 substrates were analyzed, with a minimum of 5 particles per substrate, at every scanning angle. Scanning the incident beam from 29.25° to 31.50° with respect to the horizontal axis, the average velocities were plotted against the interrogating angles and shown later in Figure 3.4.

3.4.3. Comparison Between Bimetal and Single Gold Configuration

Launching the incident beam at the SPR angle, the motions of the beams were recorded. Shown in Figure 3.3(a-c) are snapshots of a particle propelling on a 40nm silver – 10nm gold metallic layer, while Figure 3.3(d-f) depicts particle propulsion on a 50nm gold configuration. Vividly, the velocity for the polystyrene bead was faster when employing the bimetallic film configuration. Comparing a sample size of 100 particles, the average velocity obtained by a 40nm silver – 10nm gold configuration was $7.23\mu\text{m/s}$ compared to $2.57\mu\text{m/s}$ for a pure gold configuration.

Figure 3.3 (a-c) Particle propulsion on a 40nm Ag – 10nm Au metallic surface, taken at 3s intervals; (d-f)

Particle propulsion on a 50nm Au metallic surface, taken at 3s intervals.

Scanning the incident beam from 29.25° to 31.50° with respect to the horizontal axis, the average velocities were plotted against the interrogating angles and shown in Figure 3.4.

Figure 3.4 Average velocities of the polystyrene particles at different incident angles for the various metallic film configurations.

Evidently, the shape of the curves appeared exactly opposite to that of SPR reflectivity curves. During SP resonance, as shown in Figure 3.5 which corresponds to the single gold layer configuration, the reflectivity drops to a minimum, indicating that most of the energy has been translated to the excitation of surface plasmons.

Figure 3.5 Experimentally and theoretically obtained SPR reflectivity curves corresponding to the 0 nm Ag – 50 nm Au configuration.

The extended penetration of the SP field, coupled with the subsequent internal damping of SPs which transmutes into heat, are largely credited for the enhanced propulsion of the particles at the resonance point. Away from the peak, forward propulsion occurs fundamentally under the influence of thermal convection, hence explaining the generally lower velocities. Comparing the graphs plotted in Figure 3.4, it is apparent that the interrogation angle required to fulfill surface plasmon resonance condition increases with gold thickness. More importantly, the average velocities for the polystyrene beads increase with increasing silver constituent in the bimetallic layer, as indicated by the 2× improvement in particle velocities for the 40nm silver – 10nm gold metallic layer, in comparison to the 50nm gold configuration.

Figure 3.6 Propulsion velocities during SP resonance for all silver – gold combinations normalized with respect to the 40nm silver – 10nm gold configuration.

Figure 3.6 shows the propulsion velocities during SP resonance, for all silver – gold combinations normalized with respect to the 40nm silver – 10nm gold configuration. The somewhat similar behavior for both the 50nm gold and 10nm silver – 40nm gold configurations is due to the fact that the standardized film thickness of 50nm is not an optimized value to achieve close to zero reflectivity.

Predominantly, film thickness optimized to achieve minimum reflectivity yields maximum evanescent field enhancement. Therefore, for the various configurations, the SP evanescent fields deviate from their theoretical maximum, with a larger difference especially for the 10nm silver – 40nm gold configuration. Primarily, 50nm of overall film thickness was opportunely chosen, following the examples of most SPR sensors. With further optimization of the overall film thickness, it is expected that the 20% silver

– 80% gold combination will have a stronger enhancement than the 100% single gold film [87].

To verify that the propulsive mechanism was triggered by SPs, the polarization of the incident laser was switched from *p*- to *s*-polarization where no SP resonance is expected. In this polarization, the particles continued to move forward, though at a slower pace, Figure 3.7. It was evident that the absence of the SP evanescent field above the metal film resulted in a significantly weaker propulsive force, though forward propulsion was still possible due to thermophoresis. A similar behavior under *p*-polarization was observed, when changing the incident angle to a value that does not correspond to the SP resonance angle.

Figure 3.7 Comparison between propulsion velocities for excitation using TM and TE polarized beams.

3.4.4. Consideration of Thermal Effects

Along the horizontal axis, thermophoresis and the SP evanescent field propel the particles forward against friction. In comparison to TIR evanescent field, the attractive force associated with SP field is slightly different. As the particle moves towards the metal surface in the presence of a SP field, the effective refractive index within the field's penetration depth increases, annulling the resonance requirement. The attractive force on the particles diminishes and they float up again. An equilibrium point is reached and the particles hover at a fixed distance from the metal surface.

When the laser power is switched on, within the immediate vicinity of the incident beam spot, the vertical forces balance and the particles are thrust forward under the influence of thermal convection and the SP field in the horizontal plane, which has been theoretically analyzed to be approximately 3 times of a pure evanescent wave, when considered at a position 200nm from the surface of the metal layer. As the particles are driven across the metal surface (Figure 3.8(a) – 3.8(c)), these particles will eventually enter into a “hot spot” (Figure 3.8(d)) where they will be levitated off the surface. At this region, the SP field would have decayed severely, and convection becomes the domineering force. Referring to Figure 3.8(d), there were negligible signs of any forward movement once a particle encroaches into such a region. Instead, the images of the beads show signs of defocusing (Figure 3.8(e) – 3.8(f)), indicating that the particles are being levitated. Gravity restricts the upward flow of the particles and an intensely heated region caused by the incident laser spot prevents any further forward-movement of the dielectric body.

The up-rushing movement of fluid-body as a result of the thermal convection leads to the formation of an “imaginary” wall, thus the stagnation of the micro-beads. Turning off the laser at this moment stops the levitation process and the particle descends due to gravity (Figure 3.8(g)).

Figure 3.8 Frame-by-frame micro-graphs on the lateral and vertical motions of a micro-particle (white arrow) when propelling under excess thermal gradient.

3.5. Conclusion for Chapter 3

Due to the non-uniform laser intensity profile across a focused Gaussian beam being used in surface plasmon induced propelled guiding, a statistical average of the micro-particles' speed has to be taken at every single location over the entire homogeneous metal surface under monitoring. The average velocity for each bimetal combination, commencing from 0nm of Ag with 50nm of Au overcoat, to 40nm of Ag with 10nm of Au overcoat, at a 10nm interval of thickness, is plotted against the scanning angle using the Kretschmann interrogation methodology. While under the Transverse Magnetic mode of optical excitation for 40Ag – 10Au metal configuration, the photons coupled to the surface plasmons cause the sample particles to propel on average 2X faster than for a 0Ag – 50Au configuration. The increase in propulsion velocities are not intuitively as high due to the relatively modest, 3X, increase in the square of the horizontal Electric Field, as compared to the 20X increase in the corresponding vertical component. Experiments conducted to understand the effect of thermal forces on the propulsive force on the micro-particles reveal a rather flat variance in the average velocities when Transverse Electric mode of optical excitation is used, further emphasizing on the non-absorptive nature of thin metallic films toward S-polarized light, when plasmon generation is of interest.

4. Surface Plasmon Induced Optical Immobilization of Micro-particles

– Large Scale Organization and Parallel Selective 2-D Optical Tweezers on Plasmonic Landscape

4.1. Overview of Chapter 4

The flexibility of surface plasmon polaritons being used as a trapping means, instead of a propulsive force as shown in Chapter 3, is demonstrated in this segment. By manipulating the chamber depth of the sample well, the inherent thermal gradient generated by a metallic surface can be altered in such a way that minimal convective force is present to aid in the radial congregation of a large number of micro-particles. These particles gathered in the middle of the beam spot of the oblique incident beam further experience optically induced forces that tend to pull them towards the metal surface of the sample well. Such optical gradient forces will hold the particles at an equilibrium position together with the intervention of the surrounding thermal convective currents. To further tap the optically induced trapping capability of a plasmon wave, the surrounding metal can be reduced such that there would be minimum thermal influence on the immobilization of the micro-beads. This potentially points to the possibility of enabling parallel and selective optical traps. Preliminary experimental results are presented in this chapter for homogeneous metal configuration as well as isolated metal islands. This is to show that single trapping of particles could lead to a higher level of selectivity in particle manipulation.

4.2. Concept of SP Induced Organization

Thermal gradients are inherently present when a metal layer of interest is impinged by an optical beam. There would a transfer of photonic energy into thermal energy which aids in the optically induced propulsion phenomenon mentioned previously in Chapter 3. The convection velocity, U , in a thin chamber of thickness, l containing lateral temperature gradient, is shown by Rusconi et al [89] to be given by

$$U \approx g\alpha_0 l^2 \Delta T / \nu \quad (4.1)$$

where α_0 is the thermal expansion coefficient of the medium, ν is the kinematic viscosity, g is gravity, and ΔT is the vertical temperature gradient. Therefore, we can minimize the convection velocity, with a reduction in chamber thickness, l which has a square-relation to U . In this Chapter, the chamber depth used for large scale organization of micro-beads is reduced to about one-third ($\sim 33\mu\text{m}$) of the depth previously used. In doing so, the optical binding effect due to the surface plasmon wave would become the dominant force which would keep the micro-particles organized and in equilibrium position.

4.3. Experimental Setup

The experimental setup is altered in such a way to improve the imaging capability of the previously used system. The laser source is also changed from 1064nm to 532nm, as a result of technical difficulties faced. A green filter (Stop Line) is used to eliminate the scattering observed from the side of the micro-particle, as well as scattering off the metal surface caused by surface roughness. As seen in Figure 4.1, a lens is placed in front of the broadband illumination source to expand the incoming illumination beam, thus reducing the optical intensity.

Figure 4.1 Schematic for experimental setup to enable large scale organization of micro-particles.

Further more, an iris is placed after the lens to spatially filter the light source, thus enabling better imaging quality. As the particle sizes used in the subsequent experiments

are much smaller than in the propulsion case, an “optical zoom” capability is required of the system, without the need to change the objective lens. The use of a higher power objective lens would render the need to bring the objective closer to the sample cell, thus reducing the working distance. A pair of lenses is placed in between the aperture of the objective lens and that of the CCD. These lenses act like a telescopic arrangement, enabling the captured images to be enlarged, without the need to adjust the position of the objective lens drastically.

Literature indicates that the excitation light source needs to be narrow ($\sim 200\mu\text{m}$ diameter) therefore a beam-expander arrangement is placed at the laser outlet. Instead of expanding the original laser beam diameter of about 2.25mm, the reverse is required to attain approximately a 10 times reduction in laser beam waist.

4.4. Results Analysis and Theoretical Explanation

4.4.1. Striking A Balance Between Thermal and Optical Effects

From the previous chapter, it has become intuitive that any congregational phenomenon attributed to thermal effects would lead to an extended organization that is spatially circular or elliptically shaped. However, if the thermal forces within the sample chamber are well controlled, the mass of dielectric particles would aggregate at within the region of the beam spot. As seen in the figure below, optical micrographs of the same location are taken over a space of 10 minutes, and then examined qualitatively. Obviously, thermal convective force is required to radially congregate the particles. However, as the

temperature gradient is not excessively large, the particles would not have the tendency to gather in an exact elliptical shape – the shape of the beam spot when incident on the glass/metal interface. Instead, the beads would, upon encroaching into the beam spot region, start to be influenced by the enhanced vertically directed electric field. Such an electric field is theoretically calculated to be enhanced by about 20 times compared to a pure evanescent wave. The strong electric field would optically trap the in-coming particles as and when the binding force is sufficiently stronger than the thermo convective force. Thus as seen in Figure 4.2 below, there is no evidence of an aggregation into an elliptical arrangement.

Figure 4.2 Large scale organization of micro-particles at the same location after approximately 20 minutes.

4.4.2. Scattering Observed As a Result of Plasmonic Optical Trapping

Figure 4.3 Scattered light observed from one side of each of the optically trapped micro-particle.

Observed scattering from the side of a micro-particle under the influence of a surface wave has been reported in all of literature, similar to Figure 4.3, which has been obtained through experiments pertaining to this thesis. This scattering is seen in both pure evanescent wave – particle, as well as surface plasmon wave – particle interactions. The difference lies not only in the much enhanced axial electric field intensity when a metal layer is present, but that the characteristic of the surface wave on a plain prism for the evanescent wave generation, and that of the plasmonic wave on a metallic surface, differ. Goos-Hänchen shift experienced by an optical ray under total internal reflection refers to a lateral displacement between the point of incidence by an impinging optical ray and that of the point of reflection. Between these two points of micro-displacement, is the presence of a surface or an evanescent wave, whereby the electric field intensity of such a wave attenuates quickly axially. This evanescent wave does not experience much

attenuation in the horizontal plane, however, unlike that of the plasmonic wave. The generation of a plasmonic wave is a result of the evanescent wave causing harmonic oscillations of the surface electrons of the metallic film deposited on a bare prism. This plasmonic surface wave, however, is exponentially decaying in the horizontal plane. This decaying nature of the wave is caused by the absorptive characteristic of the metal layer.

Till this point, we have understood the way the electric field intensity of the respective surface waves behave. Let us now consider a Mie dielectric particle in the path of a surface wave, whose dimensions are much larger than the optical wavelength used. Since its dimensions are not of the same scale as the wavelength, we would expect the Mie particle to, not experience oscillations as a direct result of the nature of the surface wave. It is most likely that the electric field of the surface wave would propagate along the skin depth of the micro-particle. As it is now known that both evanescent and plasmonic waves have differing electric field characteristics, a similar Mie particle impinged by each of the surface wave would result in contrasting outcomes. It has been understood that as a result of the laterally decaying electric field of a plasmonic wave, there is a gradient force generated. This gradient force, somewhat similar in effect to the gradient force of a three-dimensional optical tweezer trap, tends to pull the dielectric particle backwards. The axially decaying electric field, which is present in both evanescent and plasmonic waves, tends to pull the particle towards the metal surface. The interplay between a lateral gradient force, vertical gradient force, scattering force, thermal convection and gravity leads to a region of equilibrium positions for an immobilized micro-bead. In the case of a pure evanescent wave, it is expected that the vertical gradient

force and forward k-vector of the electric field keep the dielectric particle moving forward and close to the prism surface.

It is understood from Chapter 2 that the penetration depth of the surface plasmon wave on top of a metallic surface lies in the range of hundreds of nanometers. This translates to trapping volumes of less than 5% for a $5\mu\text{m}$ diameter micro-bead. As a result of the presence of metal in a plasmonic setup, the influence of thermal convective force on the equilibrium position of a trapped micro-particle must not be neglected. For a dielectric particle to remain trapped at a laterally stationary position there is a subtle but non-negligible interaction between the forces present; namely scattering force, gradient force, thermal and gravity.

Figure 4.4 Frame-by-frame micro-graphs of a single micro-particle experiencing optical binding across a period of 1 second. The schematic depicts the relative position of the micro-particle with respect to the distance from the metal surface.

As shown above in Figure 4.4, several snap shots of a $5.72\mu\text{m}$ diameter micro-bead are taken over a 1-second interval. The inherent spot of light as a result of a dielectric body impinged by a surface wave and scattering of it is observed. Intuitively, the scattering occurs along the direction of the applied optical beam and propagation direction of the k -vector. The amount of light scattered is deemed relevant to the position of the dielectric particle with respect to the metallic surface, since the nearer a particle is to the metallic surface, the larger is the volume of particle within the penetration depth of the plasmonic field, and thus larger the interaction interfacial surface. As discussed previously, thermal force and gravity are at play and thus we would expect a trapped particle to be “hovering” above the metal surface. This would mean a change in brightness of the scattered light from the side of an immobilized dielectric bead, as seen in the figure above.

4.4.3. Changing from TM to TE Polarized – Termination of Plasmonic Optical Trap

As discussed in the previous section, evidence of particle – wave interaction could be obtained in the form of scattered light from the side of the particle. The generation of plasmonic wave is reviewed in Chapter 2, whereby Transverse Magnetic (TM) or P-polarized light must be obliquely incident, close to or at the angle of resonance, at the prism/metal interface. Transverse Electric (TE) or S-polarized light does not have the appropriately aligned electric field vectors to excite the electrons on the metal/air interface, and thus if applied, we should not expect any trapping phenomenon to be present. This is exactly what happened when a P-polarized beam used to hexagonally

arrange an array of dielectric beads, is switched to S-polarization. As shown in Figure 4.5, there is evidence of the tell-tale scattered light at the side of most of the arranged particles. The series of images are obtained over a period of approximately 90 seconds. However, once the beam polarization was switched, the prominent scattering is no longer seen. This is followed closely by pronounced “hovering” in the lateral plane. This coincides with the termination of the plasmonic wave, and thus no optical binding mechanism is in place. Thermal convective force is still present as a result of the incident S-polarized beam, as well as the inherent gravity acting on the particle. Therefore, we notice an increased Brownian motion and dissociation of any form of optically induced particle arrangement.

Figure 4.5 Regularly arranged micro-particles give way to a hap-hazard congregation upon switching from P-polarized to S-polarized excitation light.

4.5. Concept of Selective 2-D Optical Tweezers

By creating periodically spaced metal pads, any thermal effects could be isolated and localized; leading to minimal thermally generated radial aggregation at the center of the laser beam spot. Figure 4.6(a) and 4.6(c) give the schematic layout of a plasmonic landscape for immobilization of singular particles, as well as the fluidic flow of convection currents within the sample chamber. The dotted circle refers to the beam spot formed when a laser beam obliquely incidents at the glass/metal interface. In the case of a homogeneous metal, refer Figure 4.6(b) and 4.6(d), the Gaussian beam intensity profile would lead to a higher ambient temperature at the center of the fluid medium. This translates into a temperature gradient across the homogeneous metal, and thus the micro-particles would radially converge and self-align under thermal convective influence. Instead Figure 4.6(c) depicts the localization of thermal spots, with minimum convective current interference between adjacent metal pads which are spatially spread-out, would assist in the immobilization of an individual particle on each metal pad. The micro-beads which are in direct contact with the glass surface would be propelled by the evanescent field, in the direction of the k -vector. Thus as the particles propel forward, they might enter into the region demarcated by a metal pad.

Upon entering into the metallic region, two different forces tend to trap the bead within the metallic region. First, would be the localized heating due to the laser. Such localized heating would generate convection currents in such a way to prevent the micro-particle from drifting away from the metal pad. In effect, the extensive thermal convection

experienced in the homogeneous metal layout, has been shrunk to several micro-meter-sized regions. Second, there is the presence of the surface plasmon wave on the thin metal film that interacts with the micro-bead. Because of the decaying nature of the plasmonic wave in the vertical direction, as discussed previously, the dielectric body would be pulled towards the metal surface by the optical gradient force. And as a result of the absorptive nature of the metal, the electric field decays in the horizontal direction, causing the particle to be stabilized at the tapered end of the horizontal electric field profile. Therefore, up to ones discretion and application, the metal pads can be fabricated in an arrangement that serves the purpose of parallel selective-trapping at pre-determined locations.

Figure 4.6 Schematic depicting the concept of thermal-reduction for a plasmonic patterned landscape in (a) and (c), as compared to the conventional homogeneous metal.

4.6. Experimental Setup

Experimentally, the optical setup is similar to that of the homogeneous metal micro-particle organization, Figure 4.7, whereby a green laser (Verdi V, 532nm) is used as the excitation source. However, glass substrates holding structured metal pads will be used instead. Because of that, since the bulk of the surface of the substrate is uncoated with gold, most of the illumination light will pass through the glass substrate instead of being reflected back into the CCD. On top of that, transparent silica beads will be dispersed on top of the substrate, leading to even greater difficulty to locate and image the beads on the glass surface. To improve the quality of images captured, a dark-field imaging technique is incorporated into the system. Most of the incoming illumination is spatially filtered, so as to increase the contrast obtained from the image.

Figure 4.7 Schematic of the optical setup implemented for selective parallel 2-D optical tweezers.

4.6.1. Preparation of Plasmonic Landscape

A periodic gold pattern is designed on the surface of a microscope glass slide, with the structures fabricated using electron beam lithography technique together with a lift-off process. To enable reusability of the plasmonic structures, a 2nm thin layer of Titanium is used as an adhesion layer for between the 50nm gold and bulk glass. The intended layout is shown in Figure 4.8, with alternating sizes of metal structures; namely $3\mu\text{m}$ and $5\mu\text{m}$ square pads, with a period of $18\mu\text{m}$.

Figure 4.8 A schematic on the relative positions of the differently sized metal pads designed for 2-D optical tweezers.

The idea of having differing dimensions is to monitor the tendency of either a micro-particle would more likely be trapped by a structure of similar size or not [48]. However due to possible error in the data transfer of the dimensions of the structures drawn up by a

Computer Aided Design (CAD) program, to the e-Beam lithographic tool, the actual period between adjacent metallic pads fabricated turn out to be lesser than intended, as the respective square pads have increased in dimensions. The period has now been reduced to less than $15\mu\text{m}$. In addition, to reduce the thermal effects within the sample cell, a chamber of shorter depth ($\sim 33\mu\text{m}$) is used, similar to the previous Chapter, as compared to the chamber depth of $100\mu\text{m}$ used in Chapter 3.

4.7. Results Analysis and Theoretical Explanation

4.7.1. Limitation of Thermal Effects with Metal Reduction

A non-focused laser beam of diameter approximately $400\mu\text{m}$ is obliquely incident at the glass slide/metal interface. The beam spot is aligned such that all the metal spots fabricated are being enveloped by the laser spot region. For a start, a single metal spot of comparable size to the sample particles is used to analyze the trapping and moving of a $5.72\mu\text{m}$ silica particle. As seen from Figure 4.9(a) to Figure 4.9(h), we observe a single particle being trapped on top of a metal surface, while the surrounding particles of similar properties continue to be propelled in the direction of the k-vector. When the particle encroaches into the metallic region, optical interactions occur between the particle and the plasmonic wave. We can see in Figure 4.9(b) and Figure 4.9(c), that even if two particles of similar size vie for the same metal pad, only one of them will be able to remain on the metal structure under equilibrium. Furthermore, the particle tends to reside in a position at the tip of the metal pad. This position is due to the equilibrating effect of the thermal convective forces, a result of laser heating of the metal layer, and the gradient

pulling force of the surface plasmon wave. From Figure 4.9(i) and onwards, the polarization direction of the impinging optical beam is changed from TM to TE. As observed from Figure 4.9(i) through Figure 4.9(k), the particle that is initially trapped within the metal structure slowly dislocates itself from the plasmonic trap. In the TE mode, there are no electric field components that will be absorbed by the metal and be used to excite surface plasmons. The subsequent motion of the isolated particle is a result of thermal convective currents ejecting the particle from the confinement of the metal pad. It is thus conclusive to state that the silica particle is possibly held back via optical and thermal forces, and not as a result of surface roughness or adhesion.

Figure 4.9 (a) – (h) represent the entering and trapping of a single micro-particle on a single metal structure, under TM polarized light. As shown in (b) – (c), a single metal pad is too small to accommodate 2 micro-beads, and thus only one of them would be efficiently trapped optically and thermally. (i) – (k) show the subsequent stages when the initially immobilized micro-bead struggles to overcome the thermal barrier around it, when a TE polarized light is used.

4.7.2. Array of Localized Optical Trapping

However, when we consider the entire span of micro metallic structures being illuminated by the narrow laser beam, it is noticed that particles still tend to radially, as illustrated schematically in Figure 4.10, approach the center of the beam spot. This is despite the absence of a homogeneous metallic landscape. The particles when drifting overhead a micro metallic structure, experience a pronounced slowing down in movement compared to when they are above the bare glass surface.

Figure 4.10 Schematic showing the continual radial organization of the micro-particles over a patterned landscape, as a result of residual centralized heating effect by the large number of closely arranged metal pads.

Furthermore, the substrate made of glass would not be a good conductor of heat, thus what the micro-particles should experience while being on the surface of the glass substrate, as in a pure evanescent case, would be unidirectional propulsion. This could only point to possible optical interaction between the particle and surface plasmon wave. As reported in Section 4.6.1., the metal structures have been fabricated with less-than-optimized period. This could contribute to the still relatively high thermal convection as a

result of the closely arranged metal pieces. Figure 4.11 shows the schematic for what would be the ultimate intention for a successful selective 2-D optical trapping scenario.

Figure 4.11 Singly trapped particles on pre-determined metallic structures, depicted schematically.

4.8. Conclusion for Chapter 4

Exploiting the subtle balance between thermal effect and surface plasmon wave excitation can lead to a dominance of optical binding effect on the sample micro-particles. It has been shown that homogeneous metal surfaces induce thermal convective currents that aid in the radial congregation, on a large scale basis, of such dielectric particles, where once in the middle of the laser beam, would be pulled towards the metal surface as a result of the overbearing optical trapping force. Large scale organization of micro-particles has been achieved, including detailed analysis into the hovering motion as well as the scattered light of a typical particle when under the influence of the optical trap. To further extend the application of such optically bound force, a patterned metallic landscape has been utilized to cut down on the generation of thermal convection throughout a typical homogeneous metal surface. Metal structures of similar thickness as homogeneous surfaces, when fabricated at periodic spacing, can generate minimal heat such that convective current flow around adjacent structures would not interfere. Such an arrayed layout could bring about parallel immobilization of various micro-beads, with the location of each bead predetermined by the lithographically fabricated metal pads. However, as a result of technical difficulties, the fabricated structures (edge to edge distance of $11\mu\text{m}$) are not as optimally spaced and shaped as required. This discrepancy, as discussed in the previous section, could still have led to significant cumulative thermal convection at the middle of the arrayed structures, thereby causing only a small number of the $5.72\mu\text{m}$ dielectric micro-beads to be trapped singly, as predicted, on individual metal pads.

5. Surface Plasmon Resonance Imaging – Grayscale Calibration Technique

5.1. Overview of Chapter 5

One of the main objectives of this thesis is to show the feasibility of characterizing the reflectance intensity obtained in SPR images in grayscale. Several glucose solutions of differing refractive indices were dripped into the fabricated sample wells made of Mylar. A typical arrayed-SPR image, when displayed in grayscale, has an intensity gradient between each sample well. Each glucose sample is mixed and measured for the refractive index using a refractometer (Kyoto Electronics). Following which, each sample is imaged using SPR imaging technique to obtain the grayscale of the reflected intensity image.

5.2. Concept of Grayscale Calibration in SPR Imaging

Standard 8-bit black and white CCDs have 256 levels of intensity, ranging from 0 to 255. Optical images received by the CCDs are converted into pixilated format, with each pixel carrying a value between 0 and 255, inclusive. 255 would indicate the brightest and 0, the lowest possible intensities. Since SPR imaging works on the reflected intensity of a targeted medium, for example de-ionized water, it is expected that any increase to the refractive index of the target water, would lead to a shift in the reflected intensity, certis parabus. Thus by characterizing a database of fluidic samples with increasing refractive indices, it is possible to pin point the refractive index of a test sample, by using the

calibration curve, basing on the grayscale information received via the reflected intensity of the SPR image.

5.3. Experimental Setup

The experimental setup for SPR imaging, Figure 5.1, follows closely to the systems discussed in the previous chapters, except for the need to use an expanded beam in this case. The reason for an expanded beam is to average out the noise across the beam waist and reduce the signal-to-noise ratio of the received image, as well as to allow a wider span of interrogation of the sample plane.

Figure 5.1 Schematic for the optical setup required for SPR imaging.

5.3.1. Glucose Sample Preparation

5 glucose solutions of different refractive indices were prepared and stored and sealed to prevent evaporation and thus densification of the glucose solution; namely 1.3280, 1.3317, 1.3333, 1.3361, 1.3388 and 1.3407. 1.3280 refers to pure de-ionized solution which is used as a control. The 6 samples are labeled (1) through (6), from the sample with the smallest to the largest refractive index. Following that, each solution is manually injected, using a conventional syringe and needle, into the array of 6 1mm diameter wells, fabricated from Mylar material using CO₂ laser. The index of refraction of DI water changes at a ballpark value of $0.8 \times 10^{-4} / ^\circ\text{C}$. Therefore taking into consideration of possible temperature fluctuations in the DI water, as a result of thermal transfer from the irradiance source to the measurement surface, certain precautionary steps are taken to minimize any temperature variations of the samples. The illumination's optical path to the glass prism is blocked out before depositing of any sample, to prevent radiation over-exposure that could lead to minute yet significant temperature rise at the glass/metal interface. There is a need to calibrate each individual well because of various factors. The metal surface of each sample well differ from each other due to fabrication irregularities, surface defects, organic debris as well as uneven illumination during the surface plasmon resonance imaging process. These differences render variations in the reflected intensities received from each sample well, even though each well holds a solution of similar refractive index. After the first sample was injected in the array of wells, the interrogation laser's optical path is then unblocked. The CCD which is on standby all this while is used to capture an image. Following that, an optical wipe is used gently to absorb up the

solution, followed by a blow-dry using a hand-held blower. This is deemed a reliable method of sample removal due to the high level of stiction of water. Removal of the glass sample for cleaning is not advisable due to the sensitivity of the SPR technique towards positioning of the glass sample, as well as for image comparison purpose.

5.4. Results Analysis and Theoretical Explanation

5.4.1. SPR Array Imaging

Thus shown in Figure 5.2, with the 6 different samples numbered 1 to 6 respectively, are the SPR array images.

Figure 5.2 Wide-field SPR grayscale images of the same 6 sample wells (2 X 3) holding different fluidic samples of different refractive indices, (1) – (6).

Figure 5.3 is a more 3-D graphic alternative view of the SPR images converted using a MatLab code. The color code provides visual information of the difference in refractive indices at one glance.

Figure 5.3 3-D graphical representation of the above wide-field grayscale SPR images.

The refractive index of de-ionized water was set as the SPR target angle. Thus we should expect a relation between the refractive index as well as the reflected intensity. The images were taken using a black and white camera, and the resultant images were converted into grayscale values to obtain a relation plot, as shown in Figure 5.4, where each line corresponds to a unique sample well.

Figure 5.4 Experimental plots of received wide-field SPR imaging grayscale intensities against refractive index of the fluidic sample.

As a test of this imaging reliability, 2 test samples with known refractive indices of 1.3345 and 1.3369 are injected into either the left or right column of wells. The reflected intensities of the SPR images are obtained and evaluated. The test results are considerably good, as shown in Figure 5.4. The dotted ellipses denote the region of the graphical points of the test samples. Each labeled Trial number corresponds to the respective Well number. This experimental result shows that grayscale calibration of SPR images allows liquid samples of unknown refractive indices to be determined off the intensity plots with reasonable accuracy. The sensitivity of this technique, averaged over the 6 sample wells, is calculated to be 4.5×10^{-4} . However, as seen in the above SPR images, the image quality is relatively poor for a wide-field imaging technique. This is a result of the higher order reflected light interfering, thus generating the unique fringes observed. Another interesting feature of the SPR images is the elliptical grayscale images, as compared to the circular wells fabricated. The reason for this apparent

discrepancy has to do with the imaging aberrations incurred, e.g. field of curvature, by the optical rays passing through the imaging optics.

5.4.2. SPR Narrow-Field Imaging

5.4.2.1. Homogeneous Target – Fluidic Medium

In order to improve on the image quality of an SPR image, a technique employed is to use a narrow illumination spot by inserting an iris in the experimental setup, between the beam expander and the first reflector. Figure 5.5 shows how a spatially filtered SPR image looks.

Figure 5.5 A typical narrow-field SPR grayscale image obtained.

It can be seen that all the fringes have been removed and what is obtained is a high quality SPR image. Another calibration work is done using this technique on sample Well number 1. The same 6 solutions of different refractive indices are injected one after another to obtain the spatially filtered SPR image as shown in Figure 5.6.

Figure 5.6 Narrow-field grayscale SPR images obtained across 6 samples with different refractive indices, (a) – (f).

It can be seen that any variance in refractive index can be distinctly observed compared to the previous wide-field method. Figure 5.6(a) to Figure 5.6(f) corresponds to the sample number 1 to 6, respectively. Figure 5.7 is the MatLab converted 3-D graphical representation of sample Well 1 holding sample solution number 1.

Figure 5.7 3-D graphical representation of the above narrow-field grayscale SPR images.

Figure 5.8 Cross sectional intensity plots of the reflected intensities obtained from the SPR images received in the previous Figure 5.6.

Figure 5.8(a) to Figure 5.8(f) is the cross sectional intensity profiles of the SPR images in Figure 5.6. We can see that there is a much better signal to noise ratio. The cross sectional intensity profiles are compiled, averaged and then plotted against the refractive indices as shown in Figure 5.9. The sensitivity of this technique, averaged over the 6 sample wells, is calculated to be 2.6×10^{-4} .

Figure 5.9 Experimental plots of received narrow-field SPR imaging grayscale intensities against refractive index of the fluidic sample.

The gradients of the plots show a high level of similarity as the wide-field technique however, in order to perform an array SPR imaging method using spatial filtering technique, the sample or sample well sizes have to be at least in the micro-meter scale.

5.4.2.2. Heterogeneous Target – SU8 Structures

In an attempt to incorporate SPR imaging with surface plasmon induced optical manipulation, preliminary imaging work on structures to test the image resolution has to be carried out. Shown in Figure 5.10, is an SPR image of a set of circular SU8 photoresist structures fabricated via contact mask lithography technique.

Figure 5.10 Narrow-field SPR images of lithographically fabricated SU8 circular structures.

The structure diameters are given as follow, from left to right - $300\mu\text{m}$, $240\mu\text{m}$, $200\mu\text{m}$, $160\mu\text{m}$, $120\mu\text{m}$, $100\mu\text{m}$, $80\mu\text{m}$, $60\mu\text{m}$ and $40\mu\text{m}$. The above structures are fabricated on top of a typical 1mm thick microscope glass slide coated with a layer of 50nm of gold. The structures are hard-baked after photoresist development to ensure their mechanical stability. The working mechanism of SPR imaging of rigid structures goes like this. The array of SU8 structures are covered with a droplet of water, so as to create an ambient of refractive index of water of 1.3328. This would normally result in a dark SPR image received, however, with structures of much higher refractive index adhered to the surface of the sensing surface, namely the metal layer; we should expect spots of higher reflectivity which represent the SU8 structures in the midst of a dark background. Certain observations about the SPR image are made, such as the difference in intensity across the image received and the dark halo around each apparent circular structure. In order to remove the interference fringes, the spatially filtered method is used. However, due to the combined lateral distance taken up by the structures to be larger than the laser spot, there is an uneven distribution of laser power over the entire exposed region. It can quite obviously be deduced that the beam was incident around the location of structure (c) and (d), thus the higher grayscale intensity.

Figure 5.11 Cross sectional intensity profile of the narrow-field grayscale SPR image of the previous Figure 5.10.

Figure 5.11 gives the cross sectional profile of the grayscale intensity of the SPR image. It can be noticed that in order to increase the signal to noise ratio of such an image, a higher power beam spot with uniform power distribution would be desirable.

Figure 5.12 3-D graphical representation of the above narrow-field grayscale SPR image of the SU8 structures.

Figure 5.12 gives a 3-D view of the SU8 structures, further emphasizing on the non-uniformity of the illumination region.

5.5. Concept of Morphological Mapping In Grayscale Using SPR Imaging

By tapping the high level of sensitivity towards the effective refractive index sensed by the surface plasmon interrogative field, it is possible to employ the plasmon field to present the variations in the effective refractive index sensed over a thin volume, in the form of an image. As presented in the earlier sections, the surface plasmon image would be in grayscale format. However, when a foreign body such as a spherical polystyrene bead is to be present in the thin volume, as a result of the intrusion with the physical presence of the poly-bead, the effective refractive index of the thin volume has now been altered. Furthermore, since the physical shape of the bead follows a surface curvature from the single point of contact between the polystyrene particle and the metallic sensing surface, it is intuitive to note that less and less of the polystyrene material resides in the thin volume, as we move away from the middle of the sphere. Because of this varying change in refractive index per unit volume of the thin region, we should notice gradual changes in the reflected grayscale intensities across the entire image, mimicking the surface curvatures of the foreign body.

5.6. Experimental Setup

Figure 5.13 Schematic showing the SPR imaging setup for particle-morphological imaging.

Similar to the previous section on SPR array imaging, an expanded HeNe beam of uniform and low cross sectional intensity is used as the interrogating source. Instead, further monitoring of the presence of the polystyrene particles is done via an additional CCD erected above the sample well, with a broadband illumination source, refer to Figure 5.13. Magnification is made variable by including a simple lens after the dichroic mirror, where the dichroic mirror works as a reflector for the illumination light source, and yet allows the reflected light from the metal coated surface to pass through. In this SPR particle imaging experiment, large $73.7\mu\text{m}$ polystyrene beads of refractive index 1.591, are used, such that the obtained SPR image would be much larger than the lateral resolution capable of SPR imaging technique, which falls in the region of $2\mu\text{m}$ to $3\mu\text{m}$.

5.7. Results Analysis and Theoretical Explanation

By using a heavy and large polystyrene particle, the polymeric body would sink rapidly to the bottom of the sample chamber. This is to enhance the rate of experiment, as well as to ensure that the particle would be residing on the imaging surface. No adhesion layer is used in this experiment such that this method would be in-line with the intended surface plasmon enabled-propulsion, isolation and imaging. A diluted mix of polystyrene particles in DI water is dispensed into a sample chamber made from Mylar. By making use of the microscopic setup, an isolated bead is located and the resulting surface plasmon image is obtained.

Figure 5.14 MatLab plotted 3D graphical image of the obtained grayscale SP image. The pronounced center region denotes the presence of the polystyrene body.

Figure 5.14 shows the 3D plot of the reflected intensity of the surface plasmon image as a result of the presence of a polymeric body.

Figure 5.15 2D profiles of the Real (solid line) and Normalized (dotted line) reflected intensity plots against the Y-Axis Cross Sectional Profile of Figure 5.14.

Figure 5.15 shows the raw-data plot of the reflected intensity (grayscale) as well as the normalized reflected intensity against the cross sectional profile. Both plots show good reflectance contrast between the particle and the surrounding ambient. By making use of a simple SP penetration depth calculation, the expected SP image should be in the range of $7\mu\text{m}$ to $8\mu\text{m}$. This is consistent with the estimation of the magnification of the SP image obtained.

Figure 5.16 Grayscale Surface Plasmon image (best viewed in e-format) of the 73.7 μm polystyrene micro-particle and the schematic estimation of the lateral image obtained via penetration depth calculation.

Figure 5.16 presents the grayscale image obtained through the surface plasmon image technique. As per Literature [40], the obtained image contains a whitish region enveloped by a sea of darkness. This is to be expected since the ambient holding the micro-bead is water and the surface plasmon interrogation angle and refractive index employed, coincides with that of DI water.

5.8. Conclusion for Chapter 5

Preliminary surface plasmon resonance imaging capability is achieved by utilizing the Kretschmann prism coupling configuration. To increase the signal-to-noise ratio of the received SPR image, a beam-expanded, 632.8nm Transverse Magnetic polarized light is used as the interrogation source. By implementing the grayscale calibration method, a higher level of sensitivity can be achieved for every difference in the refractive index, where currently a sensitivity of 4.5×10^{-4} is attainable on a customized laboratory-grade setup. Higher orders of refracted light interfere in the far-field, thus leading to streaks of fringes in the wide-field array SPR images. As averaging technique is employed to evaluate the resultant reflected intensity in grayscale for each and every sample well, the exceptionally bright and dark fringes, which correspond to the constructive and destructive interferences respectively, would play a part in off-setting the actual reflected intensity of the targeted sample medium. A typical narrow-field SPR image does not have interference fringes as a result of the elimination of most of the higher orders of light. As such, the narrow-field SPR imaging method can be used for higher sensitivity and image quality, with a slight increase in sensitivity of more than 1.5X, at 2.6×10^{-4} , compared to the array imaging configuration. It is believed that to improve further on the sensitivity would be to apply uniform illumination across the sample well. To prepare for real-time SPR imaging of a sample particle, rigid SU8 cylindrical structures are lithographically fabricated as test structures. The relatively low lateral resolution limit for this laboratory setup could be due to the lack of a suitable test structure fabrication mask, the poor illumination consistency as well as possible incomplete development and removal of the

photoresist thus leading to an enlarged structure. Following that, heavy and large polystyrene beads of diameter $73.7\mu\text{m}$ are deposited on top of the imaging surface in DI water ambient. This is to ensure that such heavy particles would sink to the bottom of the well under the influence of gravity and encroach into the penetration depth of the surface plasmon field. Grayscale image of the reflected intensity of an isolated micro-particle is obtained, including cross sectional analysis of its profile. This grayscale SPR image depicts the surface curvature or morphology of the surface of the particle nearest to the imaging surface. The low image contrast, however, could be a result of the relatively small difference between the refractive indices of the polystyrene (refractive index 1.591) sample and the ambient DI water.

6. Conclusion and Future Work

6.1. Conclusion

The average velocity for each bimetal combination, commencing from 0nm of Ag with 50nm of Au overcoat, to 40nm of Ag with 10nm of Au overcoat, at a 10nm interval of thickness, is tabulated and plotted against the scanning angle using the Kretschmann configuration. The increase in propulsion velocities are not intuitively as high due to the relatively modest, 3X, increase in the square of the horizontal Electric Field, as compared to the 20X increase in the corresponding vertical component. Experiments are conducted to understand the effect of thermal forces on the propulsive force on the micro-particles, where when under TE polarized light interrogation the particle velocities are relatively constant, further emphasizing on the non-absorptive nature of thin metallic films toward TE-polarized light, when plasmon generation is of interest.

Optically induced thermal convection aid in the radial congregation of dielectric particles. Once in the middle of the laser beam, the particles would be pulled towards the metal surface as a result of the overbearing optical trapping force. Large scale organization of micro-particles has been achieved, including detailed analysis into the hovering motion as well as the scattered light of a typical particle when under the influence of the optical trap. Patterned metallic landscape can be utilized to cut down on the generation of thermal convection throughout a typical homogeneous metal surface. Metal structures of similar thickness as homogeneous surfaces; have been fabricated at periodic spacing, generating minimal heat such that convective current flow around adjacent structures

would not interfere. However, as a result of technical difficulties, the fabricated structures are not as optimally spaced and shaped as required. This discrepancy could still have led to significant cumulative thermal convection at the middle of the arrayed structures, thereby causing only a small number of the sample dielectric micro-beads to be trapped singularly, as predicted, on individual metal pads.

Preliminary surface plasmon resonance imaging capability is achieved by utilizing the Kretschmann prism coupling configuration. An expanded HeNe 632.8nm laser is used as the interrogation source. By implementing the grayscale calibration method, a higher level of sensitivity can be achieved for every difference in the refractive index, where currently a sensitivity of 4.5×10^{-4} is attainable on a customized laboratory-grade setup for wide-field SPR imaging. However, a typical narrow-field SPR image does not have interference fringes as a result of the elimination of most of the higher orders of light, and as such, the higher sensitivity by 1.5X and the better image quality. Rigid SU8 cylindrical structures are lithographically fabricated as test structures to investigate into the lateral resolution obtainable, with the SU8 structures immersed in ambient de-ionized water.

By using surface plasmon imaging on the presence of a polymeric micro-bead, an intensity plot derived from the image mimics the bottom-surface curvature, representing a non-uniform across sectional profile. The curvature of the intensity plot hints at the curved surface of the micro-particle, thus such an imaging technique can be used, in future, to derive a bottom surface morphology of the particle in question.

6.2. Recommendation for Future Work

Shrinkage of the physical dimensions of the coupling prism and the use of laser diodes would lead the path towards device miniaturization. A quadruple faceted right-angled prism, with the top surface open for insertion of a disposable metal-coated glass substrate, could allow incorporation of two differing-wavelength laser optical paths orthogonally, with one focused beam taking the role of immobilizer, while another expanded beam takes the role of the imager. The use of two lasers of different wavelengths would ensure no optical interference at the common spot on the substrate/metal interface.

A CMOS device at the end of the expanded imaging beam could read the reflectance output, and simple software could be used to analyze the received data and communicate it with the user on a basic dot-matrix display interface. Micro-fluidic chambers could be linked to the imaging platform, performing the role of storage cells for samples and even cleaning solvents, if necessary.

Another possible direction would be to investigate further into the relation between the electrons' characteristics on the skin depth of the thin metal layer on top of the glass substrate, and the coupling photons. When light impinges on a metal surface, it is widely known that like a semiconductor, a current is induced due to the second order nonlinear optical effect – photon drag [90]. Under the surface plasmon resonance phenomenon, each absorbed photon generates a plasmon at the metal/dielectric interface. Each plasmon

is energetically well-defined and has a uniquely consistent momentum. These surface-bound plasmons convert into hot electrons due to intraband quasi-particle excitation [53]. The momentum carried by these resonant electrons creates a current flow, while those electrons at non-resonance get converted into thermal energy.

External application of electric current across or along intended propagation direction of the propelled micro-particles might bring about an interesting effect on the behavior of the loosely-bound surface electrons of the metallic film. Since surface plasmon effect is due to the resonant oscillation of surface electrons via the coupling of photonic energy to the surface-bound electrons, there is all likelihood that by influencing the electronic oscillations, the usual particle propulsion and particle immobilization would be affected by this action. Of course, minute alterations in the material temperature would have to be suppressed such that any increase in the metal film thickness, as a result of continuous illumination of the sample surface, could be minimized, and thus reduce any shifting experienced by the surface plasmon reflectivity curve.

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