NANOGAP EMBEDDED SILVER GRATINGS FOR SURFACE PLASMON ENHANCED FLUORESCENCE

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ABSTRACT

Plasmonic nanostructures have been extensively used in the past few decades for applications in sub-wavelength optics, data storage, optoelectronic circuits, microscopy and bio-photonics. The enhanced electromagnetic field produced at the metal and dielectric interface by the excitation of surface plasmons via incident radiation can be used for signal enhancement in fluorescence and surface enhanced Raman scattering studies. Novel plasmonic structures have shown to provide very efficient and extreme light concentration at the nano-scale in recent years. The enhanced electric field produced within a few hundred nanometers of these surfaces can be used to excite fluorophores in the surrounding environment. Fluorescence based bio-detection and bio-imaging are two of the most important tools in the life sciences and improving the qualities and capabilities of fluorescence based detectors and imaging equipment remains a big challenge for industry manufacturers. We report a novel fabrication technique for producing nano-gap embedded periodic grating substrates on the nanoscale using a store bought HD-DVD and conventional soft lithography procedures. Polymethylsilsesquioxane (PMSSQ) polymer is used as the ink for the micro-contact printing process with PDMS stamps obtained from the inexpensive HD-DVDs as master molds. Fluorescence enhancement factors of up to 118 times were observed with these silver nanostructures in conjugation with Rhodamine-590 fluorescent dye. These substrates are ideal candidates for a robust and inexpensive optical system with applications such as low-level fluorescence based analyte detection, single molecule imaging, and surface enhanced Raman studies. Preliminary results in single molecule
experiments have also been obtained by imaging individual 3 nm and 20 nm dye-doped nanoparticles attached to the silver plasmonic gratings using epi-fluorescence microscopy.
1. INTRODUCTION

1.1 Plasmonics

Plasmonics is the study of optical phenomena in the vicinity of metal surfaces on the nanoscale. Due to the diffraction limit imposed on light owing to its wave nature, photons cannot be confined to regions less than half the wavelength of light. However, in the presence of metal nanostructures, the photons can couple to surface charge oscillations known as ‘surface plasmons’. This coupling or so called “resonance” enables the localization of electromagnetic energy on the nanoscale, thereby trapping light in volumes as low 1 nm$^3$ [1]. By confining light to such small volumes on the nano-level, researchers have made optical wave guiding and imaging possible beyond the diffraction limit [2, 3]. Recent studies of novel metal nanostructures have unraveled a plethora of applications that utilize the near-field localization of light for signal enhancement [4, 5] as well as signal propagation [6]. Fluorescence spectroscopy has been used extensively in the past few decades for research in the biomedical sciences, clinical diagnostics, and commercial biological and chemical threat sensors [7-9]. However, there has been a growing need to improve the contrast levels in fluorescence images and the limit of detection for fluorescence based biosensing. The advent of plasmonics in recent years has provided some of the answers required to improve the quality of fluorescence microscopy and spectroscopy through surface patterning and novel geometries [10, 11]. The enhanced electromagnetic field produced by plasmonic
nanostructures can be used to couple light to fluorescent dye molecules in the immediate environment thereby providing extreme signal multiplication necessary for detecting trace quantities of biomolecules tagged with fluorophores [10, 12]. Although high powered lasers can be used to excite the fluorophores, they require additional focusing optical components and high operational costs as compared to well-fabricated metallic nanostructured platforms that provide the same effect using a conventional broadband light source. Plasmonic nanostructures also provide an increase in the quantum yield of weakly fluorescent molecules, decrease the lifetimes of fluorophores and increased photostability [8, 13].

To this day, a myriad of novel plasmonic nanostructures have been developed and fabricated for applications such as surface plasmon fluorescence enhancement, surface enhanced Raman studies, data storage, optoelectronic circuits, and diffraction-limited microscopy [3, 14]. For instance, both 1-D and 2-D silver grating structures have been fabricated and shown to provide considerable signal enhancements using epi-fluorescence microscopy [15, 16]. Metallic nanogap bowties as well as bow tie arrays have been fabricated using e-beam lithography and used to observe individual fluorescent molecules attached to the surface owing to extreme electromagnetic field enhancement using confocal microscopy [13].

Although these platforms are highly efficient and structured precisely, they involve very high cost productions for various lithographic techniques and reactive-ion etching systems [17-19]. On the contrary, commercially available optical media have drawn the attention of many researchers in the past few years for a cheap and effective
alternative to expensive plasmonic grating nanostructures because of their mass production in the market[20, 21]. CDs, DVDs and other optical discs have periodic gratings in the form of tracks on the polycarbonate substrate that can be coated with metal films to form plasmonic metal gratings [22-24]. A disadvantage with this method though, is that these polycarbonate surfaces have to be tuned to obtain sharp resonance peaks by chemical etching methods which have issues with reliability and reproducibility [22]. Hence, there is still a need to develop a less time-consuming and low cost fabrication procedure for the mass production of plasmonic substrates that can be used for bio-sensing and high contrast imaging using fluorescence microscopy.

1.2 Thesis overview

This study explains the fabrication, characterization, and the applications of nano-gap embedded plasmonic gratings. These novel nanostructures were fabricated using conventional soft-lithography methods [25] and used for fluorescence enhancement from fluorophore molecules. The extreme field enhancement produced by these plasmonic nanostructures was also used for surface-enhanced Raman scattering (SERS) studies and imaging individual fluorescent nanoparticles.

The following chapter describes the basics of surface plasmons from an electromagnetism point of view using Maxwell’s equations. A detailed analysis of both propagating surface plasmons (SPs) and localized surface plasmons is presented. An extensive explanation of gratings coupled Surface plasmon resonance (GC-SPR) is shown to reveal the basics of surface plasmon and photon coupling. An explanation of the
combined effect of plasmonic gratings and plasmonic nano-gaps is also provided based on previous studies. The basics of fluorescence and finite difference time domain (FDTD) numerical methods are discussed in this chapter as well.

Chapter three contains all the specifics and details about the sample preparation, fabrication and characterization. The reflectance setup used to characterize the plasmonic gratings is described as well as the surface topographies obtained using atomic force microscopy (AFM) and scanning electron microscopy (SEM) are discussed. The spontaneous formation of nano-gaps is also explained briefly.

Chapter four goes on to present the data obtained for fluorescence enhancement from the nano-gap embedded gratings as well as the SERS enhancement observed when comparing to flat surfaces and rough metallic surfaces. Single 3 nm and 20 nm Rhodamine-doped organosilicate nanoparticles (DOSNP) nanoparticles [26] were assembled on the nano-gap embedded gratings and preliminary data has been obtained for imaging individual fluorescent nanoparticles using epi-fluorescence microscopy and the extreme field enhancement from the novel plasmonic structures. Numerical simulation results performed using FDTD are also presented to make a comparison between nano-gaps on flat surfaces and nano-gaps on grating surfaces.

The final chapter summarizes the important findings of this study and emphasizes the use of this plasmonic nano-structured substrate as a cost effective and convenient biological and chemical threat sensing platform.
2. THEORY

2.1 Surface Plasmons

Surface plasmons are collective charge oscillations induced at a metal-dielectric interface in the presence of an external electromagnetic field [27]. These charge density oscillations themselves exhibit electromagnetic character and can be thought of as surface waves propagating along the metal surface. Surface plasmons were first discovered by R. H. Ritchie in 1957 [28] and later studied in detail by Otto [29], Kretschmann [30], and Raether [27]. Surface plasmons can be resonantly coupled to the incident photons under certain specific conditions of wavelength, incident angle, and the optical properties of the metal and the surrounding dielectric. The surface plasmons after coupling to the photons form surface plasmon polaritons (SPPs) and this phenomenon is known as “surface plasmon resonance” (SPR). The coupling of photons to the surface plasmons leads to increased field intensity at the interface due to the evanescent nature of the surface plasmon electric field and has been used extensively in SPR based biosensors as well as for efficient excitation of fluorophores [31, 32]. Hence, the signal for the same light source can be enhanced by using a plasmonic substrate as opposed to using a substrate which does not support surface plasmons.

2.1.1 Dispersion relation of Surface Plasmons

The electromagnetic behavior of surface plasmons (SPs) can be derived using Maxwell’s equations which are as follows [33]:

\[ \nabla \times \mathbf{E} = -\mu_0 \frac{\partial \mathbf{H}}{\partial t} \]

\[ \nabla \times \mathbf{H} = \epsilon_0 \epsilon_r \frac{\partial \mathbf{E}}{\partial t} + \mathbf{j} \]

\[ \nabla \cdot \mathbf{E} = \rho \]

\[ \nabla \cdot \mathbf{H} = 0 \]
\[ \nabla \cdot \mathbf{D} = 0 \]
\[ \nabla \cdot \mathbf{B} = 0 \]
\[ \nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t} \]
\[ \nabla \times \mathbf{H} = -\frac{\partial \mathbf{D}}{\partial t} \]  
\[ (2.1) \]

Where \( \mathbf{D} \) is the electric field displacement \((\text{C/m}^2)\), \( \mathbf{B} \) is the magnetic field strength \((\text{N/A/m})\), \( \mathbf{E} \) is the electric field \((\text{V/m})\), and \( \mathbf{H} \) is the magnetic field \((\text{A/m})\).

**Figure 2.1** The electric field lines of surface plasmons at a metal-dielectric interface. The presence of an external EM field induces surface charge oscillations which form surface waves showing electromagnetic behavior as well. These surface plasmon waves are evanescent in nature showing concentration of field closest to the interface.

The electric and magnetic quantities are related to each other through the material equations:

\[ \mathbf{D} = \varepsilon_0 \varepsilon(\omega) \mathbf{E} \]
\[ \mathbf{B} = \mu_0 \mu(\omega) \mathbf{H} \]  
\[ (2.2) \]

Where \( \varepsilon_0 \) is the electric permittivity of free space, \( \mu_0 \) is the magnetic permeability of free space, \( \varepsilon(\omega) \) is the dielectric constant, and \( \mu(\omega) \) is the relative permeability. Considering isotropic and homogeneous materials, both \( \varepsilon(\omega) \) and \( \mu(\omega) \) are functions of the angular frequency.
frequency of the external electric field. Firstly, the solution to Maxwell’s equations considering a plane wave is:

\[ E(r, t) = E_0 e^{i(k \cdot r - \omega t)} \]  \hspace{1cm} (2.3)

Where \( r \) is the position vector in space, \( t \) represents the time, \( E_0 \) is the electric field amplitude and \( k \) is the wavevector. Here, \( k \) and \( \omega \) are dependent on each other through the photonic dispersion relation [34]:

\[ \frac{\omega^2}{k^2} = \frac{1}{\mu_\omega \mu(\omega) \varepsilon_0 \varepsilon(\omega)} \]  \hspace{1cm} (2.4)

Now, using the identity \( c^2 = 1/\varepsilon_0 \mu_0 \), and assuming a nonmagnetic media \( \mu(\omega) = 1 \). The photonic dispersion relation simplifies to:

\[ k = \frac{\omega}{c} \sqrt{\varepsilon(\omega)} \]  \hspace{1cm} (2.5)

Figure 2.2 Schematic showing a TM polarized wave incident on a metal-dielectric surface with no electric field component along the y-axis.

Now, let us consider an external EM plane wave incident at an interface, such that there exists a discontinuity at the boundary due to the presence of two different optical media. Only a TM polarized electric field (x-z plane) with a component in the z-
direction can generate surface plasmons since the electron oscillations in this plane experience the discontinuity at \( z = 0 \) (Figure 2.2). Suppose a TE polarized electric field (x-y plane) was incident upon the interface, no surface plasmons would be generated since there is no discontinuity in this plane. Hence, surface plasmons modes can only be observed using TM polarization of the incident plane wave [27]. Let us now consider the electric and magnetic fields in two different mediums separated by a boundary as shown in Figure 2.2. The electric field can be represented by the equations:

\[
E_1 = \begin{bmatrix} E_{x1} \\ 0 \\ E_{z1} \end{bmatrix} e^{i(k_{x1}x + k_{z1}z - \omega t)}
\]

\[
E_2 = \begin{bmatrix} E_{x2} \\ 0 \\ E_{z2} \end{bmatrix} e^{i(k_{x2}x - k_{z2}z - \omega t)} \tag{2.6}
\]

Where the indices 1 and 2, refer to the quantities at \( z < 0 \) and \( z > 0 \) respectively. The corresponding magnetic field can be calculate using Equation 2.1, and is given by:

\[
H_1 = \begin{bmatrix} 0 \\ H_{y1} \\ 0 \end{bmatrix} e^{i(k_{x1}x + k_{z1}z - \omega t)}
\]

\[
H_2 = \begin{bmatrix} 0 \\ H_{y2} \\ 0 \end{bmatrix} e^{i(k_{x2}x - k_{z2}z - \omega t)} \tag{2.7}
\]

Now, imposing the field continuity conditions for \( \mathbf{E} \) and \( \mathbf{H} \) parallel to the interface, we get:

\[
E_{x1} = E_{x2}
\]

\[
H_{y1} = H_{y2}
\]

and therefore,

\[
k_{x1} = k_{x2} = k_x
\]
Now, inserting (2.6) and (2.7) in equation (2.1), we obtain:

\[ k_{x1} H_{y1} = \frac{\omega}{c} \varepsilon_1 E_{x1} \]  \hspace{1cm} (2.8)

\[ k_{x2} H_{y1} = \frac{\omega}{c} \varepsilon_2 E_{x2} \]  \hspace{1cm} (2.9)

Dividing, (2.7) by (2.8), we get the relation:

\[ \frac{k_{x1}}{k_{x2}} = -\frac{\varepsilon_1}{\varepsilon_2} \]  \hspace{1cm} (2.10)

From (2.9), we can say that the surface plasmon solutions only exist if \( \varepsilon_2 \) and \( \varepsilon_1 \) have opposite signs as the left hand side is always positive, and is only possible in the case of a metal and a dielectric interface. The total wavevector \( k_i \) can be used to relate the \( k_z \) and \( k_x \) components and using (2.5) in the two different media as follows:

\[ k_{z_i}^2 = |k_i|^2 - k_{x_i}^2 = \left( \frac{\omega}{c} \right)^2 \varepsilon_i - k_{x_i}^2 \]  \hspace{1cm} (2.11)

Since the x components of the wavevector are equal in both media, we can now use (2.10) along with (2.11) leading us to:

\[ \left( \frac{\omega}{c} \right)^2 \varepsilon_1 - k_x^2 = \left( \frac{\varepsilon_1}{\varepsilon_2} \right)^2 \left[ \left( \frac{\omega}{c} \right)^2 \varepsilon_2 - k_x^2 \right] \]

From the above equation, we achieve the final plasmonic dispersion relation [27]:

\[ k_x = \frac{\omega}{c} \sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}} \]  \hspace{1cm} (2.12)

If we consider a metal-dielectric interface with \( \varepsilon_1 = \varepsilon_d \) (real) and \( \varepsilon_1 = \varepsilon_m \) (complex) with a negative real part and a small positive imaginary part. This implies that \( k_x \) is also a complex quantity and so is \( k_z \). If we go back and re-examine the electric and magnetic field terms (equations (2.6) and (2.7)), the z-component of the field in the dielectric for a complex \( k_z \) will be:
\[ e^{i\left((k_z^\prime + ik_z^\prime\prime)z - \omega t\right)} = e^{i(k_z^\prime z - \omega t)} e^{-k_z^\prime\prime z} \]

Where \( k_z^\prime\prime \) is the imaginary part and \( k_z^\prime \) is the real part. This represents a wave decaying exponentially in the z direction with a decay length of \( 1/k_z^\prime\prime \). Similarly, the \( E_x \) component and \( H_y \) component will also show evanescent characteristics along their respective axes.

The surface plasmon waves therefore will propagate in the x-direction bound to the surface, and decay exponentially as they travel. These surface plasmons can therefore be thought of as non-radiative waves with propagating lengths on the order of a few microns in most cases. Sometimes, these are also referred to as surface plasmon polaritons (SPPs).

### 2.1.2 Surface Plasmon Resonance

Looking at the dispersion relation for surface plasmons more closely, the dielectric permittivity of the metal can be expressed as \( \epsilon_m = \epsilon_m^\prime + i \epsilon_m^\prime\prime \). For all noble metals in the visible range, \( |\epsilon_m^\prime\prime| << |\epsilon_m^\prime| \) and the value of the surface plasmon wavevector \( k_x \) will always exceed the value of the photon wavevector in a certain dielectric (Figure 2.3).

![Figure 2.3](image-url)

**Figure 2.3** The dispersion relation of a photon in air (blue line), and the dispersion relation of a surface plasmon in air. For the same optical frequency, the surface plasmon wavevector is always greater than the photon wavevector. Therefore, additional optical momentum is needed to couple a free photon to a surface plasmon.
Therefore, to see the coupling between surface plasmons and photons, both energy as well as optical momentum matching conditions should exist. The inequality in equation 2.13 prevents free photons from coupling directly to the surface plasmons. Thus, to achieve coupling between light and surface plasmons at a particular angular frequency or specific wavelength of light, we need to provide additional optical momentum to the incident light to reach the value of $k_{SP}$ and achieve surface plasmon resonance (SPR).

2.2 Grating-Coupled SPR & Metallic Nano-gaps

2.2.1 Plasmonic Gratings

A common way of exciting surface plasmons on a metal surface using photons is through the use of prism coupling [35]. The incoming photon is incident through a high refractive index prism with a thin metal film at the prism base (Figure 2.4). Here, the photon wavevector increases by the value of the refractive index of the prism ($n_p$), and at a specific angle of incidence, the x component of the photon wavevector will equal that of the surface plasmon as shown by the equation below [29, 30]:

$$k_{SP} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}} = n_p \frac{\omega}{c} \sin \theta_{SP} \tag{2.14}$$

Where, $\theta_{SP}$ is the surface plasmon resonance angle for a specific wavelength and at this incident angle, the incoming photons can couple to the surface plasmons and achieve surface plasmon resonance.
Two different prism configurations can be used to couple light to surface plasmons on a thin metal film. In the Otto configuration (Figure 2.4 (a)), incident light is reflected at the base of a prism which has a thin dielectric film and an infinitely thick metal film deposited on the other side of the dielectric film [29]. At angles greater than the critical angle for the prism-dielectric interface, total internal reflection takes place creating an evanescent field at the prism base. This evanescent field can tunnel across the dielectric layer and excite the surface plasmons at the metal and dielectric interface. The Kretschmann [30] configuration essentially swaps the positions of the metal and dielectric from that of the Otto configuration (Figure 2.4(b)). Light is now incident at a metal surface through the high-index prism and couples to the surface plasmons under total internal reflection conditions as described before. The problem with both these methods is that the metal and dielectric layer thicknesses are very critical parameters for the configurations to function properly.

Figure 2.4 (a) Otto configuration and (b) Kretschmann configuration for coupling of incident light to the surface plasmons at a metal and dielectric interface.
Another proficient way of providing additional optical momentum to incoming light is through the use of metal gratings [36, 37]. Gratings are one-dimensional periodic arrays that are characterized by the grating pitch $\Lambda$, which is the distance from peak to peak or trough to through in the periodic pattern, and the grating height, $h$, is the distance from the bottom to the top of the structure (Figure 2.5).

![Figure 2.5](image_url)

**Figure 2.5** A plasmonic grating structure with a grating period of $\Lambda$, and a grating height $h$. In an incoming photon will experience multiple diffraction orders and for certain multiples of $G$, additional optical momentum can be provided to the $x$-component of the incident light and achieve wavevector matching.

An incoming photon incident on a metal grating will get diffracted due to the periodic pattern. The total field can be obtained by constructive interference of the individual scattered waves resulting in multiple diffraction orders [38]. Now, if the in-plane component of the $m^{th}$ diffraction order matches with that of the surface plasmons on the metal surfaces (Figure 2.5), the incoming photon can be coupled to the SP mode. After achieving SPR, the non-radiative SPs are transformed into radiative photons at the right excitation conditions, leading to near-field enhancement and localization of electromagnetic energy on the nanoscale [27].
The grating vector $\mathbf{G}$ for periodic metal gratings based on the grating pitch can be defined as:

$$\mathbf{G} = \frac{2\pi}{\Lambda}$$

The grating vector is perpendicular to the grating grooves and is in the $x$-direction as shown in Figure 2.3. It is clear that multiples $m$ of the grating vector can be added to the reflected light, and for $m = 1$, the diffracted order can provide additional optical momentum to the incoming $k_{\text{photon}}$. Using this additional optical momentum provided by the grating vector and the $x$-component of the incident light, i.e., $k_{\text{photon}} \sin \theta$, the incoming light can excite the surface plasmons on the metallic grating surface.

$$k_{SP} = \frac{\omega}{c} \sqrt{\varepsilon_m \varepsilon_d} = \frac{\omega}{c} \sqrt{\varepsilon_d \sin \theta_{SPR} + mG} \quad (2.15)$$

At a particular incidence angle, $\theta_{SPR}$, the momentum matching conditions will be satisfied for $m=1$ and the resonance condition will be achieved (Equation 2.15). It is also important to remember that the photon-plasmon coupling is only possible for TM polarized light due to the transverse magnetic nature of the surface plasmons. This near-field coupling of light can be measured directly using a reflectance setup for far-field reflected intensity as a function of the angle of incidence or the wavelength. Figure 2.6 shows the reflectance curve measured for a silver grating with a grating pitch of 400 nm and a grating height of 60 nm at a wavelength of 532 nm (TM-polarized). Here the sharp dip in reflectance at 15.9° shows the conversion of the incident light into surface plasmons and vice-versa, proving that this is the surface plasmon angle or $\theta_{SPR}$. This
phenomenon can also be referred to as grating coupled – surface plasmon resonance (GC-SPR).

### 2.2.2 Metallic nano-gaps

Plasmonic gratings are ideal substrates for near-field concentration applications as they avoid the inclusion of tedious optics such as prisms for exciting surface plasmons. Grating structures are also easily manufactured using a myriad of patterning techniques that are common to most nanotechnology facilities and labs.

![Graph: Reflectance (measured) as a function of the incident angle for TM polarized 532 nm light for silver grating with a grating pitch of 400 nm and a grating height of 60 nm. The SPR angle is denoted by a sharp dip in the curve at $\theta_{\text{spr}} = 15.9^\circ$.](image)

**Figure 2.6** Reflectance (measured) as a function of the incident angle for TM polarized 532 nm light for silver grating with a grating pitch of 400 nm and a grating height of 60 nm. The SPR angle is denoted by a sharp dip in the curve at $\theta_{\text{spr}} = 15.9^\circ$.

Another commonly used configuration for near-field concentration of electromagnetic energy is the metallic nano-gap. A metallic nano-gap with a lateral width on the order of tens of nanometers illuminated by an external EM field can provide a high intensity of localized fields where field continuity conditions concentrate the surface charges into a very small area. This “crowding” of electrical charge at sharp
nano-gap edges or ultra-sharp metal tips is known as the “lightning-rod effect” [39, 40]. The electron oscillations in this case do not constitute a propagating surface wave (as in the case of a metal film), but rather exhibit localized, non-propagating nature. Coupling an incident photon to such a nanostructure can be achieved by selecting the resonant wavelength which depends on the shape, size, and dielectric environment of the metallic nano-gap [41, 42]. This phenomenon observed in metal nanoparticles assemblies and metallic nano-gaps is termed as “localized surface plasmon resonance” (LSPR) [43].

One can now envision a nanostructure that utilizes the combined effect of both the periodic metal gratings and the metallic nano-gap. In this case, the propagating surface plasmon polaritons (SPPs) travel as surface waves with high field intensity towards the metallic nano-gap where the sudden field discontinuity causes “extreme crowding” of the surface charges leading to very high field intensities [42, 44-48]. The field enhancement within the gap can be expected to be on the order of $|\varepsilon_m/\varepsilon_d|$ owing to electromagnetic boundary conditions at the interface [49]. Hence, an individual nano-gap on a flat film will show a field enhancement of $|\varepsilon_m/\varepsilon_d|$, but a nano-gap embedded in a grating surface should show $|\varepsilon_m/\varepsilon_d|$ times the electromagnetic enhancement due to the coupling of surface plasmon polaritons. However, it is also important to remember that this enhancement is dependent on the size and dimensions of the nano-gap itself. It has been shown experimentally and computationally that the field enhancement increases as the nano-gap width decreases [50, 51]. Since these structures combine both the localized surface plasmons (LPs) of the metallic nano-gaps as well as
the SPPs from the metal gratings, the extreme field intensities can be used to excite fluorescent molecules in the vicinity enabling the detection of very low concentrations of antibodies and proteins tagged with fluorophores.

### 2.3 Fluorescence

Fluorescence is a three-stage optical transition process that occurs in certain molecules known as fluorophores or fluorescent dyes \([8, 52]\). This optical process can be illustrated using a simple electronic-state diagram called the Jablonski diagram (Figure 2.7). An incoming photon with energy \(h\nu_{EX}\) excites the electrons from the ground state \(S_0\) to the excited state \(S_1\) at room temperature. Once in the excited state, the electrons will relax to a lower vibrational energy level \(S_{1v}\), without emitting radiation due to the collisions between molecules in condensed phases. This conversion takes place on the order of \(10^{-12}\) seconds. After reaching the lower vibrational levels in \(S_{1v}\), the electrons radiatively transition to \(S_0\), a process which occurs on the order of \(10^{-8}\) seconds. The molecule now emits photons with energy \(h\nu_{EM}\), which is lower in energy than the incoming photon. This difference in energy is termed as the Stokes shift and can be identified using appropriate optical filters in the detection scheme.

![Jablonski diagram](image)

**Figure 2.7** Jablonski diagram showing fluorescence as a three step process (1) Excitation from \(S_0\) to \(S_{1v}\) through an incoming photon. (2) Relaxation from \(S_{1v}\) to \(S_1\) through multiple vibrational energy levels. (3) Emission at a higher wavelength due to the transition from \(S_1\) to \(S_0\).
Besides the spontaneous radiative decay from $S_1$ to $S_0$, there are other relaxation processes that are non-radiative in nature. One of the most influential non-radiative decay processes in the case of fluorophores present close to metal surfaces is “quenching”. The excited state of the fluorophore can couple to the electronic states of the bulk metal and relax by essentially heating the metal. Hence, quenching leads to a loss in the fluorescence emission intensity is one of the drawbacks when using metal plasmonic substrates. When placing fluorophores on silver grating for instance, one needs to take into account both the quenching as well as the evanescent field build-up at the interface.

The interaction between a metal and dye can be treated similar to a dipole-dipole interaction described well by the Förster model [53]. For the interaction between two point-like dipoles, the efficiency of energy transfer is directly proportional to $d^6$, where $d$ is the separation distance. However in the case of a infinitely extended metal plane, the integration over whole are compensates by a factor of $d^2$ [53]. The emission efficiency ($\eta_{em}$) of the dye in this case is given by the following equation:

$$\eta_{em}(d) = \frac{I(d)}{I_{\infty}} = \left[1 + \left(\frac{d_F}{d}\right)^4\right]^{-1} \quad (2.16)$$

Where $d$ is the distance between the metal and the fluorophore, $I(d)$ is the emission intensity at a finite distance $d$, $d_F$ is the Förster radius, and $I_{\infty}$ is the emission intensity where the fluorophore will not interact with the metal. The Förster radius is defined as the separation between the metal surface and the fluorophore where the emission efficiency is 0.5. For most dye molecules, the Förster radius is about 5 nm ($\eta_{em} = 0.5$) and hence, the fluorophores should be at least 10 nm away from the metal surface to avoid
quenching [54-56]. This distance will however vary depending on the optical properties of the metal, the fluorophore emission, and the local environment [52, 55, 57]. In case of thin fluorophore films on a plasmonic grating surface around 30 nm thick, the first 10 nm of the film will experience considerable quenching [35, 58], whereas the top 20 nm will see the full effect of the evanescent field enhancement. We will see this behavior in section 4.1 where fluorescence enhancement is observed using thin fluorophore films in the vicinity of nano-gap embedded plasmonic gratings.

2.4 Finite difference time domain (FDTD)

FDTD is one of the most widely used algorithms used for simulating various nanostructures in the presence of an external optical field, although its applications extend to both microwave and radio frequency regimes [59-61]. The FDTD method essentially provides a rigorous solution to the Maxwell’s equations without including any other theoretical approximations. Since Maxwell’s equations are most commonly expressed in differential form, the FDTD method solves these by discretization via central differences in both time and space and then numerically solves all the equations using computing power [62]. The basic concept is based on a grid or mesh concept developed by Kane Yee in 1966 [63]. The whole computational domain is broken down into a grid with points spaced $\Delta x$, $\Delta y$, and $\Delta z$ (Figure 2.8) apart and time is also broken down in steps of $\Delta t$ (time step). The E field components are then computed at times $t = n\Delta t$ and the H fields at times $t = (n+1/2) \Delta t$, where $n$ is an integer representing the compute step. For example, the E field at a time $t = n\Delta t$ is equal to the E field at $t = (n-1)$
\[\Delta t\] plus an additional term computed from the spatial variation, or curl, of the H field at time \(t\).

**Figure 2.8** In a Yee cell of dimension \(\Delta x, \Delta y, \) and \(\Delta z\), the H field is computed at points shifted one-half grid spacing from the E field.

This method results in six equations that can be used to compute the field at a given mesh point, denoted by integers \(i, j, k\). These are as follows [63]:

\[
\begin{align*}
H_{x(i,j,k)}^{n+1/2} &= H_{x(i,j,k)}^{n-1/2} + \frac{\Delta t}{\mu \Delta z} \left( E_{y(i,j,k)}^n - E_{y(i,j,k-1)}^n \right) - \frac{\Delta t}{\mu \Delta y} \left( E_{z(i,j,k)}^n - E_{z(i,j-1,k)}^n \right) \\
E_{x(i,j,k)}^{n+1} &= E_{x(i,j,k)}^n + \frac{\Delta t}{\varepsilon \Delta y} \left( H_{z(i,j+1,k)}^{n+1/2} - H_{z(i,j,k)}^{n+1/2} \right) - \frac{\Delta t}{\varepsilon \Delta z} \left( H_{y(i,j,k+1)}^{n+1/2} - H_{y(i,j,k)}^{n+1/2} \right) \\
H_{y(i,j,k)}^{n+1/2} &= H_{y(i,j,k)}^{n-1/2} + \frac{\Delta t}{\mu \Delta x} \left( E_{z(i,j,k)}^n - E_{z(i,j,k-1)}^n \right) - \frac{\Delta t}{\mu \Delta z} \left( E_{x(i,j,k)}^n - E_{x(i,j-1,k)}^n \right) \\
E_{y(i,j,k)}^{n+1} &= E_{y(i,j,k)}^n + \frac{\Delta t}{\varepsilon \Delta z} \left( H_{x(i,j+1,k)}^{n+1/2} - H_{x(i,j,k)}^{n+1/2} \right) - \frac{\Delta t}{\varepsilon \Delta x} \left( H_{z(i,j,k+1)}^{n+1/2} - H_{z(i,j,k)}^{n+1/2} \right) \\
H_{z(i,j,k)}^{n+1/2} &= H_{z(i,j,k)}^{n-1/2} + \frac{\Delta t}{\mu \Delta y} \left( E_{x(i,j,k)}^n - E_{x(i,j,k-1)}^n \right) - \frac{\Delta t}{\mu \Delta x} \left( E_{y(i,j,k)}^n - E_{y(i,j-1,k)}^n \right) \\
E_{z(i,j,k)}^{n+1} &= E_{z(i,j,k)}^n + \frac{\Delta t}{\varepsilon \Delta x} \left( H_{y(i,j+1,k)}^{n+1/2} - H_{y(i,j,k)}^{n+1/2} \right) - \frac{\Delta t}{\varepsilon \Delta y} \left( H_{x(i,j,k+1)}^{n+1/2} - H_{x(i,j,k)}^{n+1/2} \right)
\end{align*}
\]

These equations are iteratively solved in a leapfrog manner, alternating between computing the E and H at subsequent \(\Delta t/2\) intervals. To obtain a stable computational
simulation, one should take into consideration the Courant condition relating the
temporal and spatial resolution parameters [64]:

\[
 c\Delta t < \frac{1}{\sqrt{\left(\frac{1}{\Delta x^2}\right)^2 + \left(\frac{1}{\Delta y^2}\right)^2 + \left(\frac{1}{\Delta z^2}\right)^2}} \tag{2.17}
\]

Where \(c\) is the speed of light in vacuum and the other parameters have been defined
previously.

2.4.1 Boundary Conditions

Boundary conditions are a very important part of the FDTD algorithm as they define
the behavior of the computation at the edge of the domain. In this work, three different
boundary conditions have been used [65]:

A. Perfectly Matched Layer (PML)

A PML condition at the boundary essentially adds an additional thickness to the
boundary that can be defined using the PML thickness parameter. The purpose of this
PML layer is to act as an absorber and reduce the amount of unwanted reflections back
into the domain when the EM field interacts with the boundary. The reflectivity is
usually set to very low values so that the boundaries do not interfere with the actual
computational domain for a particular configuration.

B. Periodic Boundary Condition

This boundary condition is very important when simulating periodic structures and
works very well for the case of metal gratings. This condition states that a field leaving
the boundary on one end will re-enter the domain on the other end, and can be
expressed as:
Where $\Lambda$ represents the periodicity of the pattern in the x-direction and $k$ defines the phase difference between the field entering and the field leaving at the two boundaries. Using the periodic boundary condition instead of a PML reduces the computation time significantly as the size of the computational domain has been reduced drastically.

C. Perfect electrical Conductor (PEC)

This boundary condition is helpful when simulating materials such as highly reflective metals. For our simulation purposes, we will be simulating thick metal films that are almost entirely reflective in the visible range, and hence we can use the PEC boundary condition which acts as a very thin PML layer with very high reflectivity. This helps in reducing the simulation time significantly by reducing the total computational domain size.

2.4.2 Launch Conditions

In this study, we have used a continuous wave (CW) excitation to simulate the silver grating patterns as well as the nano-gap embedded gratings. The CW excitation is used when the steady state operation of a system is required [65]. This excitation is essentially a plane wave sinusoidal excitation multiplied by two envelope functions:

$$g(t) = \alpha(t)\beta(t)\sin\left[\frac{2\pi}{\lambda} t + At^2\right] \quad (2.19)$$

Where $\alpha$ and $\beta$ are the envelope functions that provide a steady ramp up (from 0 to 1) and a steady ramp down (from 1 to 0), respectively. The factor $A$ produces a chirped excitation and $\lambda$ is the wavelength of the incident excitation.
FDTD simulations were used in this study to study the near-field distributions of the nano-gap embedded silver gratings and to compare the theoretical results with the experimental data obtained for the fabricated substrates and will be discussed in section 4.2.
3. EXPERIMENTAL

3.1 Grating fabrication

As mentioned previously, periodic grating structures on the nanoscale are usually fabricated using two-beam interference photolithography [66] or e-beam lithography [67, 68]. Another way of manufacturing diffraction gratings is using an ultra-sharp diamond tip to mechanically etch a glass substrate by vibration damped machines but this procedure is prone to environmental factors such as temperature and pressure [69]. A comparatively fast and cheap way to obtain a grating pattern is to use commercially available CDs and DVDs. These optical media have periodic corrugation in the form of thousands of tracks scribed on the polycarbonate surface [20, 22]. This pattern can easily be replicated onto a polydimethylsiloxane (PDMS) elastomeric stamp [70] which can later be used to print grating patterns on different substrates such as glass and/or silicon using a suitable polymer as the “ink”. The process essentially combines the use of a store-bought HD-DVD as the master mold [71] (which can be purchased for a few dollars) along with conventional micro-contact printing [72].

3.1.1 Cleaning the Master Mold: HD-DVDs

i. Commercially available HD DVD-Rs (Memorex, Inc.) were dissected into two halves to reveal the grating pattern on the inside of the polycarbonate substrate.

ii. The grating pattern is usually coated with a dye recording layer. This dye layer was removed by rinsing with IPA (2-Propanol, Sigma Aldrich, 99%) thoroughly.
iii. This was followed by drying it completely to evaporate the IPA using dry nitrogen.

3.1.2 Formation of PDMS stamps

To replicate the grating pattern present on the HD-DVDs, the following procedure was used:

I. A PDMS solution was made using a 5:1 ratio by weight of the polymer to the cross-linking agent (Sylgard® 184 silicone elastomer kit available through Dow Corning, Inc.)

II. This solution was poured over the HD-DVD grating pattern and left overnight for the PDMS mold to be formed with the same grating pattern.

III. The grating pattern after being transferred to the PDMS was later used as a stamp to print on a glass/silicon substrate.

3.1.3 PDMS μ-contact printing

Micro-contact printing is a very common form of soft lithography where a PDMS elastomeric stamp is used to print some sort of “ink” on to another surface. For our fabrication purposes, after forming the PDMS grating stamp using an HD-DVD as mentioned above, the PDMS stamp is spin coated with the “ink” for 30 seconds at 3000 rpm. The “ink” used for this process is a 3% by weight solution of polymethylsilsesquioxane (PMSSQ) polymer in ethanol (Sigma Aldrich, 99%). PMSSQ (Techneglas Technical Products, GR-650F) is an organo-silicate polymer that consists of CH₃ groups (non-polar and hydrophobic) and OH groups (polar and cross-linking) in a
silicon backbone. PMSSQ is chosen as it exhibits stability and robustness at room temperatures, although it is susceptible to drastic fluctuations in relative humidity. Therefore, it is important that the fabrication process (Figure 3.1) be carried out in a clean room environment with stable relative humidity conditions - lower than 20%.

After the spin coating is complete, the PDMS stamp is immediately placed upside down on to a glass or silicon surface. These glass and silicon substrates have been already cleaned using a regular AMD (Acetone-Methanol-Deionized water) wash followed by a drying step with nitrogen and then placed on the hot plate at 100°C for 2 minutes to evaporate all the solvents completely. The stamp is left on the glass for a few seconds to transfer the pattern through surface adhesion and then peeled off after a few seconds using tweezers. After removing the PDMS, the transferred PMSSQ grating is visible with the naked eye as it diffracts the white light from the surroundings into vibrant colors. The thickness of the printed grating is around 100 nm which is verified by depositing the same film on a silicon substrate instead of the PDMS stamp and using the Cauchy dispersion for modeling the acquired ellipsometric data.

A very important by-product of this printing procedure is the formation of discontinuities or “nano-gaps” in the PMSSQ gratings. The origin of these nano-gaps is believed to arise from the mechanical strain [73, 74] developed in the thin PMSSQ film (approximately 100 nm measured using ellipsometry) after the spin coating step. Even a slight bending of the elastomeric PDMS stamp produces a tensile strain in the thin PMSSQ film, which then leads to the formation [75] and transfer of the nano-gaps in the printing step. Since the fabrication procedure is done manually (by hand), we can expect
a random distribution of the nano-gaps embedded in the gratings varying from stamp to stamp, depending on a myriad of factors such as the force with which the PDMS is peeled off, the direction of peeling as well as the extent of bending of the PDMS stamp [76]. Section 3.2 contains a more detailed characterization of the surface and details of the nano-gaps embedded in the gratings.

![Diagram of PDMS, PMSSQ, and substrate with steps i, ii, iii, iv.]

**Figure 3.1** (i) Fabrication of the PDMS stamp from a master mold (HD DVD-R, Memorex Inc.) (ii) Spin coating of PMSSQ on the elastic PDMS stamp. (iii) Printing the stamp on a Si substrate (iv) Peel of the stamp to complete the transfer of the PMSSQ grating.

A similar fabrication procedure was performed for a flat PDMS elastomeric stamp obtained by pouring PDMS in a plastic petridish, one which did not have the HD-DVD pattern transferred on its surface. Figure 3.2 illustrates the production of nano-gaps due to the tensile strain in the PMSSQ thin film on top of the PDMS stamp in both the flat PDMS stamp and the HD-DVD patterned PDMS stamp. The manual peeling of the PDMS using a pair of tweezers introduces tensile strain in the PDMS stamp as well as the PMSSQ, leading to the formation of nano-gaps during the printing step.
Figure 3.2 (a) Spin-coated PMSSQ thin film on a PDMS stamp. (b) μ-contact printing on a glass or silicon substrate followed by peeling of the PDMS stamp using tweezers. (c) The formation of nano-gaps as a result of the tensile strain developed in the film during peel-off. A zoomed-in cross-sectional view of the fabricated nano-gap within the PMSSQ film is shown for clarity.

3.1.4 Metal deposition on PMSSQ gratings

Now that the PMSSQ gratings have been successfully transferred on a rigid substrate, a thick metal film can be deposited on top to obtain a sub-wavelength plasmonic grating. The metal depositions were carried out using an AJA RF Magnetron sputtering system. The sputter process was carried out at room temperature, 100 Watt RF power, 20 sccm Argon flow, and a deposition pressure of 4 mTorr. First, a 2 nm layer of titanium (0.2 Å/sec) was deposited on the substrates which serves the purpose of better adhesion between the glass and silver as well as the PMSSQ and silver. Following
this, a 100 nm layer of silver (100 W RF, 4 mTorr, 1.5 Å/sec) was deposited to complete the fabrication of the plasmonic gratings.

![Figure 3.3](image.png)

**Figure 3.3** With the use of a commercially purchased $10 HD-DVD and a commonly available polymer (PMSSQ), high-quality nanofabrication of periodic gratings can be achieved.

### 3.2 Surface characterization

Characterizing the topography of the printed PMSSQ gratings is important to realize the efficiency and accuracy of the micro-contact printing process as described above. It is also imperative to determine the exact grating pitch and height of the fabricated nanostructure, as that will provide the optimal conditions for light coupling to the gratings and the embedded nano-gaps. Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) are two of the most widely used techniques in modern-day surface characterization of nano-structures. A combination of these two techniques was used to image the surface topographies of the sub-wavelength grating patterns as well as the nano-gaps embedded in the grating patterns.

#### 3.2.1 AFM Characterization

Atomic Force Microscopy, a type of scanning probe microscopy (SPM), was first developed in 1986 by Binnig, Quate and Gerber [77]. This technique offers a very high
resolution of imaging down to the nanometer level, almost 100 times better than
diffraction-limited optical microscopy [78, 79]. The AFM setup consists of a cantilever
with an ultra-sharp tip with a radius of curvature on the order of a few nanometers,
usually fabricated from silicon or silicon nitride [80, 81]. The tip when brought in close
proximity to the sample being scanned results in the deflection of the cantilever owing
to the various forces that come into play, such as contact forces, van der Waals forces,
electrostatic forces, chemical bonding, etc. This deflection is measured using a laser spot
reflected from the cantilever into a photodiode. Based on the signal acquired at the
photodiode, a topographic image can be constructed for the sample under
consideration. Of the different modes of operation available for the AFM, the tapping
mode provides the least amount of damage to the sample surface and is also accurate
enough to measure sub-wavelength features. In the tapping mode, or the “AC mode”,
the cantilever oscillates near its resonant frequency using a piezoelectric element. This
piezoelectric actuator uses a feedback loop to control the height of the tip above the
sample by maintaining fixed cantilever oscillation amplitude as various parts of the
sample are scanned. A commercial AFM 5500 system (Agilent Technologies) with Vista
silicon tip (k = 40 N/m, Resonant frequency = ~ 295 kHz, length = 125 um, width = 40
um, tip height = 14 um, tip radius <= 10nm) was used to scan the fabricated samples in
AC mode. Figure 3.4 shows the surface profile of the cleaned HD-DVD polycarbonate
surface. This profile shows that the HD-DVD surface has a gratings pitch of Λ = 401 nm
and a grating height of h = 80 nm. The replicated pattern on the PDMS stamp is shown
in Figure 3.5 showing a grating pitch of Λ = 401 nm and a grating height of h = 65 nm.
The lowering in the grating height can be attributed to the replication of the HD DVD pattern by the PDMS mixture which only replicates up to a certain depth due to the viscosity of the PDMS solution and the imposed aspect ratio condition. The next figure (Figure 3.6) shows the surface profile of the PMSSQ gratings printed on a glass substrate. As expected, the pattern is well replicated showing a grating pitch of $\Lambda = 401$ nm and grating height of $h = 65$ nm. After the silver deposition, the grating pitch, $\Lambda = 401$ nm and $h = 64$ nm follow the PMSSQ grating pattern very consistently, although the average surface increases due to grain formation in metal films deposited using sputtering (Figure 3.7).

**Figure 3.4** (a) Three-dimensional topography of a Memorex HD DVD-R polycarbonate surface after proper cleaning with IPA showing a scan area of 5 $\mu$m by 5 $\mu$m. (b) The surface profile for the image shown above averaged over an area of 128 pixels using Gwyddion (SourceForge.net – Free AFM data analysis software). This profile shows a grating pitch of $\Lambda = 401$ nm and grating height of approximately $h = 80$ nm.
Figure 3.5 (a) Three-dimensional topography of the PDMS stamp replicating the HD-DVD pattern showing a scan area of 5 μm by 5 μm. (b) The surface profile for the image shown above averaged over an area of 128 pixels using Gwyddion (SourceForge.net – Free AFM data analysis software). This profile shows a grating pitch of $\Lambda = 401$ nm and grating height of approximately $h = 65$ nm.

Figure 3.6 (a) Three-dimensional topography of the PMSSQ gratings printed on a glass substrate showing a scan area of 5 μm by 5 μm. (b) The surface profile for the image shown above averaged over an area of 128 pixels using Gwyddion (SourceForge.net – Free AFM data analysis software). This profile shows a grating pitch of $\Lambda = 401$ nm and grating height of approximately $h = 65$ nm.
Figure 3.7 (a) Three-dimensional topography of the silver gratings deposited using RF sputtering at room temperatures showing a scan area of 5 μm by 5 μm. (b) The surface profile for the image shown above averaged over an area of 128 pixels using Gwyddion (SourceForge.net – Free AFM data analysis software). This profile shows a grating pitch of Λ = 401 nm and grating height of approximately h = 64 nm.

The cross-sectional layout of the final structure can be visualized in a more simplistic manner as shown in Figure 3.8. The AFM measurements prove that the fabricated plasmonic gratings display high quality nanostructures with a precise grating pitch as well as the grating height.

Figure 3.8 Cross-sectional diagram of the fabricated plasmonic nanostructure using simple soft lithography procedures.
3.2.2 SEM Characterization

Although force microscopy works fairly well for measuring surfaces with lateral resolution on the order of a few hundreds of nanometers, the SEM serves a better purpose for resolving features that are 20 nm or less. Scanning electron microscopy uses a focused beam of high energy electrons (on the order of keVs) that are incident upon the sample of interest [82]. The interaction of these high energy electrons with the specimen leads to the reflection of high-energy electrons by elastic scattering (backscattered electrons), emission of secondary electrons due to inelastic scattering, and electromagnetic radiation, each of which are measured using different specialized detectors [83]. The surface topography of a sample can be obtained by looking at the intensity data from the secondary electron detector. Another important requirement for SEM imaging is the presence of a conducting surface to avoid charge buildup and proper grounding. Since the fabricated samples have a conducting silver film, we did not require any additional sample preparation for imaging. To image the nano-gaps embedded within the silver gratings, a Hitachi 4700 FE-SEM (Field-emission Scanning Electron Microscope) was used. Images were obtained using an accelerating voltage of 5000 V, an emission current of 11000 nA, and a working distance of 5200 μm under vacuum. Figure 3.8 shows a top-view of one of the nano-gaps cutting across the periodic silver grating and a lateral width of approximately 30 nm, which is not constant as it is a randomly formed nano-gap. Using image analysis tools from ImageJ and calibrating pixels of the image using the length scale provided, the width of the nano-gap can be estimated to be around 30 nm. The SEM image in Figure 3.9 is a two dimensional image and only provides feature
sizes in the x and y dimensions, and no information about the depth in the z direction. Hence, the lateral width changes for different positions along the entire length of the nano-gap.

Figure 3.9 (a) A top-view SEM image of the nano-gap embedded in the silver gratings. It is evident that the lateral width of the nano-gap changes along the entire length of the nano-gap. (b) The 2-point profile of the nano-gap along the yellow line shows a nano-gap with an approximate lateral width of 30 nm.

Figure 3.10 A three dimensional SEM image taken for the nano-gaps cutting across the silver grating taken at 5keV accelerating voltage and working distance of 4.5 mm.
3.3 Optical characterization

Reflectance measurements are a standard way for spectral and angular characterization of plasmonic gratings [84, 85]. The fabricated silver gratings were characterized using a J. A. Woollam Variable Angle Spectroscopic Ellipsometer (VASE) with air as the surrounding medium. This setup comprises of a Xenon broadband source which directs light through a monochromator via an optical fiber. The light is now incident on the silver grating sample with the gratings aligned perpendicular to plane of incidence (as mentioned before) for optimum excitation of the surface plasmons on the silver grating. The proper alignment of the grating was made possible by cutting the PDMS stamps in such a fashion that the circular tracks on the HD DVD were parallel to one of the edges. Since the direction of the circular tracks is known, we can align the gratings perpendicular to the incoming light as they are parallel to the stamp edge. The light after reflecting from the silver grating substrate reaches the detector iris (Figure 3.11) and is then passed on to a Si detector (spectral range: 200 nm – 1000 nm). The normalized reflectance curves as a function of the incident TM polarized wavelength from 500 nm – 700 nm (steps of 1 nm) for 6 different incident angles were obtained (Figure 3.12). The resonance wavelength is characterized by a sharp minimum in the reflectance curves indicating the localization of the electric field at the silver-air interface. Besides the sharp reflectance dip which denotes the resonance wavelength for a specific angle of incidence, we observe a cusp that appears for slightly lower wavelengths where the reflectance value drops as well (Figure 3.12). At the cusp, one of
the diffracted orders has a diffraction angle of 90°, which leads to a lower value of reflectivity in that region [86].

![Optical setup for reflectance using a rotary stage, monochromator, and Si detector with the gratings fixed on the vertical stage using a vacuum pump.](image)

**Figure 3.11** Optical setup for reflectance using a rotary stage, monochromator, and Si detector with the gratings fixed on the vertical stage using a vacuum pump.

![Measured reflectance spectra for the fabricated silver gratings as a function of wavelength for 6 different incident angles: θ_\text{inc} = 15°, 20°, 25°, 30°, 35°, and 40°.](image)

**Figure 3.12** Measured reflectance spectra for the fabricated silver gratings as a function of wavelength for 6 different incident angles: θ_\text{inc} = 15°, 20°, 25°, 30°, 35°, and 40°. The SPR angle (θ_{SPR}) is denoted by the sharp dip in reflected intensity at the minima. Inset: Linear extrapolation of the measured data to calculate the θ_{SPR} = 8° for 495 nm transverse magnetic light.
As an example for 495 nm TM polarized light, the value of $\theta_{\text{SPR}} = 8^\circ$ was obtained by linear extrapolation of the measured reflectance plots (Figure 3.12 inset). This value agrees very well with the resonance angle calculated using equation 2.15 and the value obtained from FDTD simulations in section 4.2. Similarly, the resonance angle for different wavelengths (Table 3.1) can be calculated from the linear fit obtained above in Figure 3.12.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Ag-Air Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>480</td>
<td>4.8</td>
</tr>
<tr>
<td>500</td>
<td>9.0</td>
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<tr>
<td>520</td>
<td>13.3</td>
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<tr>
<td>540</td>
<td>17.6</td>
</tr>
<tr>
<td>560</td>
<td>21.9</td>
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<tr>
<td>580</td>
<td>26.1</td>
</tr>
<tr>
<td>600</td>
<td>30.4</td>
</tr>
<tr>
<td>620</td>
<td>34.7</td>
</tr>
</tbody>
</table>

Table 3-1 The table above shows the SPR angle for the fabricated silver gratings and air interface for different wavelengths based on the linear fit obtained above.

It is obvious that changing the dielectric environment of the silver gratings also changes the coupling conditions for the surface plasmons as seen by the dispersion relation previously. The silver gratings were covered with a thin film of 10 µM Rhodamine-doped PMSSQ with a thickness of 31 nm and refractive index, $n = 1.41$ (@ 630 nm) which was deposited using a spin coater. The thickness and optical constants of the above mentioned film were determined using a Cauchy dispersion relation for the modeling of the acquired ellipsometric data. Reflectance measurements were now carried out for 620 nm TM polarized light varying the angle of incidence from 15° to 40° (Figure 3.13), and a decrease in the SPR angle is observed from 35° to 26°, which is expected due to the increase in the dielectric permittivity of the immediate
environment of the silver grating. The SPR angle is an important value as it leads to the optimal photon-surface plasmon coupling at a certain wavelength, producing an increase in the near-field intensity at the metal grating-dielectric interface.

![Graph showing reflectance spectra for silver gratings](image)

**Figure 3.13** Measured reflectance spectra for the fabricated silver gratings as a function of incident angle for 620 nm TM polarized light showing the decrease in SPR angle after depositing a 31 nm layer of Rhodamine-doped PMSSW film.

### 3.4 RF Sputtering vs. Thermal Evaporation

To determine the role of surface roughness on the reflectance properties of the gratings, silver films prepared using two different deposition methods were fabricated and characterized using AFM and reflectance measurements. Thermal evaporation basically involves heating the metal beyond its sublimating point in a high temperature stable tungsten boat. The metals atoms in gaseous form are then deposited on to a substrate placed above the boat. PMSSQ gratings were fabricated as described earlier and then deposited with an adhesion layer of Chromium (3 nm) and a 100 nm film of silver (30% evaporation power, 0.5 Å/sec, 2.6 X 10^-6 Torr, and room temperature). RF sputtering on the other hand utilizes the bombardment of high energy Argon ions to
extract atoms out of a metal target, following which these are deposited on substrates. PMSSQ gratings were sputtered with silver using an AJA RF Magnetron sputtering system. The sputter process was carried out at room temperature, 100 Watt RF power, 20 sccm Argon flow, and a deposition pressure of 4 mTorr. First, a 2 nm layer of titanium (0.2 Å/sec) was deposited on the substrates which serves the purpose of better adhesion between the glass and silver as well as the PMSSQ and silver. Following this, a 100 nm layer of silver (100 W RF, 4 mTorr, 1.5 Å/sec) was deposited to complete the fabrication of the plasmonic gratings.

Figure 3.14 (a) AFM micrograph of PMSSQ gratings with a 100 nm silver layer deposited using thermal evaporation and (b) a 100 nm silver layer deposited using RF sputtering.

The silver surface roughness in both cases was characterized by measuring the surface topographies using AFM (Figure 3.14) and obtaining the roughness parameters as shown in Table 3.2 using Gwyddion (free SPM analysis software). These values represent the analysis for a 5 μm x 5 μm area on the silver surface. $R_a$ represents the average roughness of the surface, $R_q$ represents the root mean square roughness, $R_t$ represents the maximum roughness height, $R_v$ represents the maximum roughness valley depth, and $R_p$ represents the roughness peak height.
<table>
<thead>
<tr>
<th>PMSSQ gratings - No silver</th>
<th>Thermal Evaporation - Ag</th>
<th>Rf Sputter - Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra</td>
<td>0.68 nm</td>
<td>3.7 nm</td>
</tr>
<tr>
<td>Rq</td>
<td>0.93 nm</td>
<td>4.5 nm</td>
</tr>
<tr>
<td>Rt</td>
<td>6.13 nm</td>
<td>25.2 nm</td>
</tr>
<tr>
<td>Rv</td>
<td>2.12 nm</td>
<td>13.4 nm</td>
</tr>
<tr>
<td>Rp</td>
<td>4.01 nm</td>
<td>11.8 nm</td>
</tr>
</tbody>
</table>

Table 3-1 A comparison of the various roughness parameters for the printed PMSSQ gratings, thermally evaporated silver on the PMSSQ gratings and RF sputtered silver on the PMSSQ gratings. RF Sputtering produces a smoother film than thermal evaporation.

It is evident from the roughness analysis that the silver filmed deposited using thermal evaporation is rougher than the one deposited using sputtering. This increase in film surface roughness can be attributed to the formation of smaller grain size in the case of thermal evaporation as opposed to the formation of larger grain size in RF sputtering caused due to increased adatom mobility [87]. To see how the roughness properties of the silver surface affect the surface plasmon resonance properties, reflectance measurements were performed in air as mentioned before for 532 nm TM polarized light as a function of the incidence angle (Figure 3.15).

![100nm Silver on PMSSQ in Ethanol Gratings](image)

Figure 3.15 A comparison of the reflectance curves for a flat silver film deposited using RF sputtering, Ag grating deposited using RF sputtering, and Ag grating deposited using thermal evaporation for 532 nm TM polarized light.
Table 3-2 (a) The SPR angle for 5 different RF sputtered silver gratings for 532 nm TM polarized light showing an average SPR angle of 15.95 degrees. (b) The SPR angle for 3 different thermally evaporated silver gratings for 532 nm TM polarized light showing an average SPR angle of 16.27 degrees.

Tables 3.3 (a) and (b) show the variation of the SPR angle for the same wavelength from sample to sample for RF sputtered silver and thermally evaporated silver, respectively. The standard deviation for different samples is close to 0.1°, which is extremely good considering the ease and cost-effectiveness of the grating fabrication procedure. The value of reflectance at the resonance angles is 0.02 units lower for the RF sputtered gratings (Figure 3.15), which indicates that the coupling is slightly more efficient. This can be attributed to the rougher silver gratings from thermal evaporation and more scattering of the incident light leading to less coupling efficiency. Hence, the smoother RF sputtered silver gratings were chosen for fluorescence studies which will be discussed in section 4.1.

3.5 High temperature treatment of PMSSQ gratings

The fabrication procedure described in section 3.1 provides a stable PMSSQ grating which maintains the periodic pattern even after sputtering or thermal evaporation of metal. However, there are certain applications that require a high-
temperature stable platform as in the case of nano-porous films [88]. Subjecting the PMSSQ gratings to a high temperature of 150 °C destroys the grating pattern due to the high mobility of PMSSQ pattern which is not cross linked at this point and is therefore unstable (Figure 3.17 (c)). To stabilize the PMSSQ gratings for high temperatures, the 3% solution of PMSSQ in ethanol (previously used as the “ink”) was cross-linked further by adding 3-aminopropyltriethoxysilane (APTES) which comprised 0.5% of the total solution volume. APTES provides better cross linking to the PMSSQ polymer due to the presence of freer hydroxyl groups. The same micro-contact printing procedure was followed to prepare gratings, and films were also prepared on flat silicon substrates for FTIR analysis, thickness and refractive index measurements. In this study, 5 different target temperatures were achieved: 150 °C, 250 °C, 350 °C, 450 °C, and 550 °C. Exposing the PMSSQ-APTES gratings to higher temperatures ensures the cross linking of the polymer matrix and hence improves the stability for other high temperature fabrication procedures. The temperature steps are explained by means of a temperature vs. time plot which were accomplished by using a furnace. Figure 3.16 shows the different temperature steps followed to reach to the target temperatures for the PMSSQ-APTES gratings. Basically, after reaching the target temperature at a ramp rate of 0.3 °C per minute, the PMSSQ-APTES gratings were maintained at the target temperature and then cooled down to room temperature by turning off the furnace.
3.5.1 AFM Characterization

AFM images were obtained using the same setup as described in section 3.2.1 for the different grating patterns after the various temperature treatment steps. The grating pitch, $\Lambda$, and the grating height, $h$, were analyzed from the topographical data obtained using the AFM images.

Figure 3.16 A graphical representation of the different temperature steps used to achieve the stability of PMSSQ-APTES gratings for 5 different target temperatures ranging from 150 °C to 550 °C.

Figure 3.17 Grating pitch and grating height as a function of the target temperature for the different temperature steps. The grating pitch remains fairly constant for different temperatures; where as the grating height reduces to 48 nm from 62 nm at 550 °C.
Figure 3.17 shows the variation of the grating pitch as well as the grating height for the different temperature steps. With increasing target temperature, the grating height remains constant until 550 °C where it falls to 48 nm. The important thing of note here is that the gratings remain stable up to a temperature of 550 °C and the periodic pattern is not destroyed (Figure 3.18).

![AFM micrographs](image)

**Figure 3.18** (a) AFM micrograph of a stamped PMSSQ-APTES grating at room temperature. (b) AFM micrograph of the same PMSSQ-APTES grating subjected to the 550 °C target temperature step described above. (c) A PMSSQ grating (no APTES) subjected to the 150 °C temperature step showing that the grating pattern has been destroyed.

### 3.5.2 Thin film Characterization

The PMSSQ-APTES thin films spin coated on to silicon substrates were subjected to the same temperature steps as the PMSS-APTES gratings mentioned above. JA Woollam VASE was used to form ellipsometry measurements from 300 nm – 1000 nm
for three different incident angles – 65°, 70°, and 75°. All the samples with different
temperature treatments were measured to study the variation in film thickness and
refractive index with increasing temperature. The Cauchy dispersion (Equation 3.1) [89]
was used to model these thin transparent films and the mean squared error value for all
the fits were in the 1-2 range (Table 3.4). Figure 3.19 shows this change in film thickness
and refractive index (@ 630 nm) as a function of the target temperature reached for the
various temperature steps explained above.

\[ n(\lambda) = A + \frac{B}{\lambda^2} \]  

(3.1)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Film thickness (nm)</th>
<th>Refractive index (n)</th>
<th>( \chi^2 )</th>
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<tr>
<td>27 C</td>
<td>113.28</td>
<td>1.429</td>
<td>2.259</td>
</tr>
<tr>
<td>150 C</td>
<td>101.24</td>
<td>1.426</td>
<td>1.135</td>
</tr>
<tr>
<td>250 C</td>
<td>104.63</td>
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</tr>
<tr>
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<tr>
<td>450 C</td>
<td>100.62</td>
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<tr>
<td>550 C</td>
<td>85.23</td>
<td>1.385</td>
<td>1.243</td>
</tr>
</tbody>
</table>

Table 3-3 The film thickness and refractive index at 630 nm for the PMSSQ-APTES films subjected to
different temperature treatments along with the mean squared error for the Cauchy dispersion fit.

![Figure 3.19](image)

Figure 3.19 The film thickness and refractive index at 630 nm for the PMSSQ-APTES films as a function of
the target temperature.
The refractive index of the film decreases with increasing temperature, and the film thickness also reduces to 85 nm from 113 nm in case of the 550 °C temperature step (Figure 3.19). This decrease in both thickness and refractive index at higher temperatures can be attributed to the burning off of the CH₃ molecules which is also evident in the FTIR spectra (Figure 3.20), where the 2975 cm⁻¹ peak that corresponds to the -CH₃ bending mode [88] decreases in intensity with increasing temperature.

3.5.3 Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was performed on all the samples subjected to different temperature treatments to study the cross linking of the PMSSQ in the PMSSQ-APTES solution. The raw FTIR spectra are shown in Figure 3.20 for different temperature treatments.

![Figure 3.20](image)

**Figure 3.20** FTIR spectra for the PMSSQ-APTES films subjected to different temperature treatments. These are offset for ease of view.
To see the degree of cross linking in the PMSSQ polymer film due to the addition of APTES and increase in temperature, the ratios of the 1035 cm\(^{-1}\) peak (Si-O-Si bridge configuration) and 1135 cm\(^{-1}\) peak (Si-O-Si cage configuration) [88] were compared for different temperatures. The measured absorbance (A) was converted into the absorption coefficient (\(\alpha\)) using the film thickness (\(t\)) measured previously as follows:

\[
\alpha = \frac{A \ln(10)}{t \ln(10)} \text{ cm}^{-1}
\]  

Equation 3.1 was derived from the Beer-Lambert law [90] which relates the absorption of light in a thin film based in the properties of the materials through which the light travels. After obtaining the absorption coefficients from the raw FTIR spectra, the 1035 cm\(^{-1}\) (peak 1) peak and the 1135 cm\(^{-1}\) (peak 2) peak were fitted using two Gaussian curves in Origin 8 Pro. The experimental and fitted data for one of the temperature treatments is shown in Figure 3.21. The ratio of the height of peak 1 to the height of peak 2 as well as the ratios of the areas under both the Gaussians was plotted as a function of the temperature (Figure 3.22).
Figure 3.21 The experimental FTIR data in the 1250 cm\(^{-1}\) – 975 cm\(^{-1}\) region fitted using two Gaussian peaks for the PMSSQ-APTEs film subjected to a target temperature of 250 °C.

<table>
<thead>
<tr>
<th></th>
<th>Area</th>
<th>Center</th>
<th>Width</th>
<th>Height</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>433075.0158</td>
<td>1027.27189</td>
<td>37.34798</td>
<td>9252.00866</td>
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<tr>
<td>2</td>
<td>580598.2435</td>
<td>1109.72791</td>
<td>80.08354</td>
<td>5784.58928</td>
</tr>
</tbody>
</table>

\[ R^2 = 0.99015 \]

Figure 3.22 The ratios of the areas and the heights of the two Gaussian peaks fitted to the experimental FTIR data. The increasing value of \(H_1/H_2\) with temperature indicates improved cross linking and stability.
From the FTIR analysis done above, it is clear that the ratio of the 1035 cm\(^{-1}\) peak to the 1135 cm\(^{-1}\) peak is increasing with rising temperature indicating the cross linking of the PMSSQ-APTES film, although the ratio of areas does not show any significant trend. The reason behind the value of the ratio \(A_1/A_2\) not showing any significant trend might be due to the poor fitting of the peaks. The appearance of a third peak at higher temperatures (Figure 3.22) might change the values significantly leading to better Gaussian fits. However, the increase in the \(H_1/H_2\) ratio ensures that there are a lot more Si-O-Si bonds that are present in the bridge configuration as opposed to the cage configuration, indicating more cross linking between polymer chains. Therefore, temperature treatments in a controlled manner lead to highly cross linked grating patterns that can be utilized in various high-temperature processes.
4. RESULTS

4.1 Fluorescence enhancement from nano-gap embedded gratings

Plasmonic gratings have been used extensively to excite the fluorophores present in the immediate vicinity of the evanescent field and achieve better limits of detection and improved imaging contrast in fluorescence microscopy \([91, 92]\). The combination of nano-gaps embedded in the plasmonic grating structure provides much higher levels of near-field concentration due to the combination of localized SPR and propagating surface plasmon polaritons as discussed in section 2.2. The evanescent field produced in the nano-gap embedded gratings is much stronger than the gratings alone. Therefore, we can expect much higher emission intensities from fluorophores present at the intersection of a nano-gap with the plasmonic grating as opposed to a grating without the presence of nano-gaps or just nano-gaps on a flat silver film.

4.1.1 Fluorescence Microscopy

The most common laboratory tool to study the phenomenon of fluorescence is the epi-fluorescence microscope \([93]\). As shown in Figure 4.1, the optical setup consists of a broadband light source which is passed through an excitation filter (optical band pass filter). After the excitation filter, light passes through a dichroic mirror which reflects the shorter wavelengths. This excitation light is incident upon the fluorescent sample through a high quality objective lens, which then emits fluorescence in an
isotropic manner. The emitted light now passes through the dichroic once again and finally through an emission filter before reaching the detector.

![Figure 4.1](image)

**Figure 4.1** Schematic representing the optical setup for a conventional epi-fluorescence microscope. The light incident on the sample is in the form of a cone of excitation which depends on the surrounding medium and the numerical aperture of the objective lens.

To study the fluorescence enhancement from the nano-gap embedded gratings, a 10 µM solution of Rhodamine 590 in PMSSQ and Ethanol was spin coated onto the substrates at 3000 rpm for 30s. This produces a thin uniform film (31 nm, n = 1.41 @ 630 nm) of fluorophore in a PMSSQ polymer matrix as discussed in section 3.3. Doping the Rhodamine dye in a PMSSQ matrix provides additional photo-stability and increases the photo-bleaching time when compared to Rhodamine in other organic solvents or DI water. Rhodamine 590 was chosen because of its high photo-stability and extensive application in biotechnology applications such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA [94]. In a traditional epi-fluorescence microscope setup, light illuminates the sample in the form of a cone of
excitation which contains all angles from $0°$ to a maximum angle $\alpha$ (Figure 3.2), given by the following equation [16]:

$$NA = n \sin \alpha \quad (4.1)$$

where, $NA$ is the numerical aperture of the objective lens used, $n$ is the refractive index of the medium between the sample and the objective, and $\alpha$ is the maximum incident angle [34, 95].

![Diagram](image.png)

**Figure 4.2** In an epi-fluorescent setup, light is incident in the form of a cone of excitation which is controlled by the numerical aperture and the surrounding environment.

For a 10x objective lens with a NA of 0.3 and air surrounding the sample, the maximum incident angle is $17.5°$ which is greater than the $\theta_{SPR} = 8°$ of the silver gratings in an air for 495 nm as shown previously via reflectance measurements. After depositing the 30 nm layer of R6G in PMSSQ, the angle further decreases as shown in section 3.3. Hence, the surface plasmons on the silver gratings can be excited using just a regular microscope setup as the $\theta_{SPR}$ is contained within the cone of excitation of a 10x objective which includes both TM as well as TE polarization states. Additionally, as seen in Figure 4.3 the absorption peak of Rhodamine is centered on 535 nm and is ideal.
to be used with a FITC filter cube set whose optical components are shown in the same figure.

Figure 4.3 (a) The chemical structure of Rhodamine 590 which is available in a reddish powdered form. The excitation and emission spectra of commercially available Rhodamine 590 (R6G) along with the optical components of the FITC filter cube set used for measuring fluorescence.

Fluorescence measurements of the samples were carried out using an Olympus BX51WI Epi-fluorescence Microscope with a 300 W Xenon white light source using a FITC filter (excitation: 460 nm – 495 nm; emission: 510 nm and beyond) and a 10x objective (Olympus-UPlanApo, NA=0.3). A high resolution CMOS camera (Orca Flash 2.8, Hamamatsu Photonics, and Internal Gain - 200) was used to obtain the images below with different exposure times. To reduce the intensity of the incident light on the samples to prevent photo bleaching, two different neutral density filters- U-25ND25 (25% transmission) and U-25ND6 (6% transmission) were used.

4.1.2 Fluorescence Data Analysis

Fluorescence image analysis was done using ImageJ (free image analysis software provided by the National Institute of Health (NIH)) software to measure the
average intensity from all the images obtained using the in-built function in the software. The adjust threshold function helps in selecting areas in an image based on a user defined threshold intensity value. The intensity of the nano-gaps was measured by using the “adjust threshold” function and analyzing just the nano-gaps to measure the average intensity selected by the enclosed nano-gap region. The tables 4.1 and 4.2 below show the average intensities obtained for all the substrates with a 10x objective and two different neutral density filters with different exposure times and the enhancement factor (EF) has been calculated using the following formula [15]:

$$EF = \frac{I_n - I_{nB}}{I_f - I_B} \quad (4.2)$$

Where $I_n$ is the fluorescence intensity with dye on the grating, $I_{nB}$ is the intensity without dye on the grating, and $I_f$ and $I_B$ are intensity with dye and without dye on glass, respectively.

![Figure 4.4](image)

**Figure 4.4** Fluorescence micrographs of (a) “nano-gaps” embedded in gratings (b) “nano-gaps” on flat silver and (c) glass slide taken with a 10x objective and a FITC filter and a 6% ND filter and 60 ms exposure time of the CMOS detector. The intensity inside the nano-gap embedded gratings is much brighter than nano-gaps on a flat film for the exact same fluorophore thin film on the surface.
Table 4.1 The above table shows the average fluorescence intensity values for different exposure times that were measured using a FITC filter, 10x air objective, Gain 200, and neutral density filter – 6% transmission.

<table>
<thead>
<tr>
<th>Exposure Time (ms)</th>
<th>nano-gaps only</th>
<th>nano-gaps Gratings</th>
<th>flat Ag</th>
<th>Ag gratings</th>
<th>glass</th>
<th>background-glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 ms</td>
<td>137</td>
<td>936</td>
<td>86</td>
<td>380</td>
<td>65</td>
<td>52</td>
</tr>
<tr>
<td>60 ms</td>
<td>173</td>
<td>1287</td>
<td>107</td>
<td>544</td>
<td>74</td>
<td>54</td>
</tr>
<tr>
<td>80 ms</td>
<td>210</td>
<td>1795</td>
<td>128</td>
<td>706</td>
<td>84</td>
<td>55</td>
</tr>
<tr>
<td>100 ms</td>
<td>258</td>
<td>2171</td>
<td>148</td>
<td>870</td>
<td>93</td>
<td>57</td>
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</table>

<table>
<thead>
<tr>
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<th>EF (NG gratings)</th>
<th>EF (ng-flat)</th>
<th>EF(flat Ag)</th>
<th>background -flatAG</th>
<th>background - Ag grating</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 ms</td>
<td>25</td>
<td>68</td>
<td>7</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>60 ms</td>
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<td>62</td>
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<td>3</td>
<td>54</td>
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<tr>
<td>80 ms</td>
<td>22</td>
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<td>2</td>
<td>56</td>
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<tr>
<td>100 ms</td>
<td>23</td>
<td>59</td>
<td>6</td>
<td>3</td>
<td>58</td>
</tr>
</tbody>
</table>

Table 4.2 The above table shows the average fluorescence intensity values for different exposure times that were measured using a FITC filter, 10x air objective, Gain 200, and neutral density filter – 25% transmission.

<table>
<thead>
<tr>
<th>Exposure Time (ms)</th>
<th>nano-gaps only</th>
<th>nano-gaps Gratings</th>
<th>flat Ag</th>
<th>Ag gratings</th>
<th>glass</th>
<th>background-glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>20ms</td>
<td>217</td>
<td>1766</td>
<td>137</td>
<td>965</td>
<td>98</td>
<td>55</td>
</tr>
<tr>
<td>40ms</td>
<td>382</td>
<td>3465</td>
<td>228</td>
<td>1832</td>
<td>128</td>
<td>63</td>
</tr>
<tr>
<td>60ms</td>
<td>527</td>
<td>3808</td>
<td>319</td>
<td>2692</td>
<td>170</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EF (gratings)</th>
<th>EF (NG gratings)</th>
<th>EF (ng-flat)</th>
<th>EF(flat Ag)</th>
<th>background -flatAG</th>
<th>background - Ag grating</th>
</tr>
</thead>
<tbody>
<tr>
<td>20ms</td>
<td>21</td>
<td>40</td>
<td>4</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td>40ms</td>
<td>27</td>
<td>52</td>
<td>5</td>
<td>2</td>
<td>64</td>
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<tr>
<td>60ms</td>
<td>26</td>
<td>38</td>
<td>5</td>
<td>2</td>
<td>73</td>
</tr>
</tbody>
</table>

Figure 4.5 Fluorescence micrographs of (a) “nano-gaps” embedded in gratings – 100% intensity – 10 ms exposure time (b) “nano-gaps” embedded in gratings – 6% intensity – 60 ms exposure time and (c) “nano-gaps” on flat silver film- 6% intensity – 60 ms exposure with a 60x water immersion objective and a FITC filter.
Figure 4.6 Enhancement factors plotted as a function of exposure time for (a) 6% ND transmission filter and (b) 25% ND transmission filter.

The different EFs for all the substrates were calculated with respect to a microscope glass slide. As mentioned in tables 4.1 and 4.2, nano-gaps embedded in gratings, grating without nano-gaps, only nano-gaps on flat silver films and flat silver films were analyzed in this study to see which substrate offers the highest fluorescence enhancement compared to glass. An important point to be made here is that the nano-gaps on flat films were fabricated using a flat PDMS stamp instead of using a periodically patterned HD DVD-R PDMS stamp. This ensures the formation of the nano-gaps due to the mechanical stress in the elastomeric PDMS but without the presence of periodic gratings. Hence, in this case we can only expect the presence of localized surface plasmons due to the presence of metallic nano-gaps as opposed to a combination of SPPs and LSPR in the case of nano-gaps embedded in the gratings. It is evident from Figure 4.6 that the enhancement factor for the all the substrates decreases slightly with increasing exposure time and hence, we can select an optimal exposure time for
maximum fluorescence enhancement. The decrease in enhancement for increasing exposure time is due to the saturation of the detector at higher exposure times, which leads to a lower average enhancement for all the substrates when compared to glass. For instance, if the intensity values of the nano-gap separated pixel regions are greater than the maximum value that can be detected (4095 in this case), then all those values will be set to 4095 and hence the average will be less than the true enhancement. Based on the above analysis, further image analysis was performed for the case of 6% ND filter and 40 ms exposure time as it yields the highest enhancement factor for all substrates.

The images obtained with the 10x objective were analyzed using ImageJ software and the nano-gaps were separated from the grating part using a threshold intensity value. Then, the “analyze particles” in-built function was used to form an outline of the nano-gaps and separate them from the background grating as shown in

![ImageJ analysis windows](image)

Figure 4.7 The two images above show the ImageJ analysis windows showing how the nano-gaps are separated from the background grating region in the image.
After separating the nano-gaps from the grating region, the mean and maximum values of the intensities in each enclosed region were obtained. The mean intensity value refers to the average of all the intensity values within the selected region, whereas the maximum intensity value represents the pixel with the maximum intensity value within the enclosed region. The software picked out 463 different enclosed regions based on the images above and the histogram for both the mean and maximum values for intensities of the enclosed region.

Figure 4.8 below shows the frequency distribution (histogram) of the maximum and mean enhancement factors found in the respective enclosed regions as calculated using equation 4.2.

**Figure 4.8** Frequency distribution for the nano-gaps embedded in silver gratings (a) Maximum Enhancement factor and (b) Mean enhancement factor. Due to the random distribution of the nano-gaps we see a distribution of the maximum and mean fluorescence intensities observed on the nano-gap embedded gratings.

Similar analysis was performed for just the silver gratings and compared to glass substrate. Figure 4.9 below shows the distribution of the maximum and also the mean enhancement factors.
Figure 4.9 Frequency distribution for the Ag gratings without nano-gaps (a) Maximum Enhancement factor and (b) Mean enhancement factor. This graph describes the variation in the fluorescence enhancement from the silver gratings in different parts of the same sample.

Again, similar analysis was performed for the nano-gaps just on a flat silver surface. Figure 4.10 below shows the distribution of the maximum and also the mean enhancement factors.

Figure 4.10 Frequency distribution for the nano-gaps on a flat silver film (a) Maximum Enhancement factor and (b) Mean enhancement factor.
Similar analysis was performed for a flat silver surface. Figure 4.11 below shows the distribution of the maximum and also the mean enhancement factors.

**Figure 4.11** Frequency distribution for a flat silver surface (a) Maximum Enhancement factor and (b) Mean enhancement factor.

Based on the image analysis performed above for the different substrates, we can compare the mean enhancement factor as well as the maximum enhancement factor for all the different substrates. Figure 4.12 shows that the nano-gaps embedded in the gratings are almost 68 times brighter than a regular glass substrate on average, and can show a maximum enhancement of up to 118 times.

**Figure 4.12** Graphs comparing the mean enhancement factors and the maximum enhancement factors of the different substrates with respect to a microscope glass slide.
The nano-gap embedded gratings with the thin fluorophore layer were also imaged using SEM as well as an optical microscope for a bright field image. Figure 4.13 shows the images of the exact same area imaged using three different techniques, which agree well with each other. The bright-field image shows the nano-gaps as dark features in a white background of the reflected silver gratings. The SEM image also provides leverage to the fact the extraordinary fluorescence enhancement seen in these samples is due to the presence of very narrow nano-gaps, which are on the order of 20 nm - 30 nm in terms of their lateral width.

Figure 4.13 (a) Fluorescence micrograph of the nano-gap embedded gratings with a thin Rhodamine-doped film on top using a 40x objective and FITC filter set. (b) Bright-field image of the exact same area using a 40x objective. (c) SEM image of the same area as in a and b showing the actual physical dimensions of the nano-gaps.

Figure 4.14 (a) Fluorescence micrograph of the nano-gap embedded gratings with a thin Rhodamine-doped film on top using a 100x oil-immersion objective and FITC filter set. (b) SEM micrograph of the nano-gaps embedded in the gratings with the Rhodamine film on top, showing a lateral width of approximately 20 nm.
A 100x oil immersion objective (Olympus-UPlanApo, NA=1.3) was also used to image the fluorescence from the nano-gap embedded silver gratings. Figure 4.14 (a) shows a very high-resolution image depicting the silver gratings and the nano-gap cutting across them at an angle. The points of high fluorescence intensity occur at the intersection of the nano-gap with the grating pattern due to the extreme field enhancement at this point. The scanning electron micrograph of the nano-gap embedded gratings with the thin Rhodamine-doped film deposited on top (Figure 4.14 (b)) shows a nano-gap lateral width of approximately 20 nm.

During the fluorescence analysis of the above samples, we also observed some regions where the nano-gaps embedded in silver gratings had the same fluorescence intensity as the surrounding grating region (Figure 4.15). These nano-gaps were further analyzed for surface topography using AFM and it was found that the lateral width of these nano-gaps was on the order of 700 nm (Figure 4.16).

![Figure 4.15](image_url) Fluorescence image of the bigger nano-gaps show that the enhancement inside these cracks is the same as on the surrounding grating region. Fluorescence image was taken with a 40x objective, FITC filter and 20 ms exposure time using the CMOS camera.
Figure 4.16 AFM scan of the same area as in Figure 4.15 showing the lateral width of the larger nano-gaps on the order of 700 nm and a depth of approximately 90 nm.

The above data proves that to observe larger fluorescence enhancements, the lateral width of nano-gaps should be on the order of 20-30 nm and not in the micro regime as shown in Figure 4.14. One probable explanation of this phenomena could be the non-existence of resonant cavity modes in such as large gap, leading to no additional field enhancement [46].

4.1.3 Fluorescence Studies with Cy5

Silver gratings were fabricated by depositing a thick 100 nm silver film on top of PMSSQ gratings using E-beam evaporation. To prevent the silver from oxidizing and to protect it from the environment, a thin layer of titanium dioxide (TiO2) was deposited on top. The thickness of the deposited TiO2 film was measured using spectroscopic
ellipsometry on a flat silver film. Spectroscopic data was collected from 550 nm – 1200 nm and for two different angles of $60^\circ$ and $70^\circ$. The data was modeled using Ag as a substrate (100 nm silver is optically almost 100% reflective), and then using the Cauchy dispersion and also an inbuilt TiO2 dispersion to fit the data. Table 4.3 below shows the thickness, refractive index and mean squared error obtained from the data analysis.

<table>
<thead>
<tr>
<th></th>
<th>thickness (nm)</th>
<th>$n$ @ 630 nm</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
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<td>CAUCHY.mat</td>
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<td>1.91</td>
<td>2.48</td>
</tr>
<tr>
<td>TiO2.mat</td>
<td>29.29</td>
<td>1.8904 + 0.0017i</td>
<td>4.99</td>
</tr>
</tbody>
</table>

Table 4-3 The measured thickness of the titanium dioxide layer on top of the silver fitted using two different dispersion models.

The surface topography of the above samples was also measured using AFM as explained before (Figure 4.17).

![Figure 4.17](image) 3D image of the TiO2 covered silver gratings deposited using E-beam evaporation along with the surface topography.
The TiO$_2$ covered gratings were also characterized using reflectance measurements to obtain the SPR wavelength at different angles (Figure 4.18).

**Figure 4.18** The SPR wavelength characterized by a sharp dip in reflectance for different incident angles. From linear extrapolation, the SPR angle for specific wavelengths can be estimated for these TiO$_2$ covered silver gratings.

To test the fluorescence enhancement from these plasmonic gratings due to field confinement on the surface, Cy5 dye was chosen. The excitation and emission spectra of the Cy5 dye along with the Cy5 filter are shown in Figure 4.19 below. This graph shows that the excitation filter is a band pass filter which will excite the gratings from wavelengths 610 nm -645 nm for which some angles are less than 18° and some are greater. Hence, we can excite the plasmons on the TiO$_2$-Ag gratings using a Cy5 filter and a 10x objective (NA = 0.3, maximum angle = 17.5°) in conjugation with Cy5 dye. The Cy5 dye was obtained from EHS.
Figure 4.19 The excitation and emission spectra of commercially available Cyanine 5 (Cy5) along with the optical components of the CY5 filter cube set used for measuring fluorescence.

For fluorescence studies, the TiO2-Ag grating samples were spin coated with a film of Cy5-PMSSQ (10 μM) in ethanol at 3000 rpm for 30s. The solution was prepared by adding 50 μL of 1 mM Cy5 in ethanol to 5 g of 1% by weight solution of PMSSQ in ethanol.

Fluorescence imaging of the samples were carried out using an Olympus BX51WI Epi-fluorescence Microscope with a 300 W Xenon white light source using the Cy5 filter and a 10x objective. A High-Res CMOS camera (Orca Flash 2.8, Hamamatsu Photonics, and Internal Gain - 200) was used to obtain the images below with different exposure times.
Fluorescence image analysis was performed as discussed earlier for the various substrates. Table 4.4 enlists all the measured intensity values for the different substrates at different exposure times.

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>nano-gaps</th>
<th>gratings</th>
<th>flat Ag-TiO2</th>
<th>TiO2 Ag gratings</th>
<th>glass</th>
<th>EF (gratings)</th>
<th>EF (NG gratings)</th>
<th>EF (NG flat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 ms</td>
<td>227</td>
<td></td>
<td>69</td>
<td>119</td>
<td>59</td>
<td>23</td>
<td>59</td>
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<td>60 ms</td>
<td>295</td>
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<td>80</td>
<td>136</td>
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<td>168</td>
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<table>
<thead>
<tr>
<th>Exposure time</th>
<th>background-glass</th>
<th>background-flat</th>
<th>background-grating</th>
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<tbody>
<tr>
<td>40 ms</td>
<td>56</td>
<td>50</td>
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<tr>
<td>60 ms</td>
<td>60</td>
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<tr>
<td>500 ms</td>
<td>172</td>
<td>105</td>
<td>105</td>
</tr>
</tbody>
</table>

Table 4-4 The above table shows the average fluorescence intensity values for different exposure times that were measured using a CY5 filter, 10x air objective, for the TiO$_2$ covered silver gratings.

Based on the image analysis performed above for the different substrates, we can compare the mean enhancement factor for all the different substrates. Table 4.4 shows that the nano-gaps embedded in the gratings are almost 60 times brighter than a regular glass substrate on average. Therefore, the average enhancement seen in this case for the nano-gap embedded gratings is less than that seen for Rhodamine which is 68 times for the mean enhancement factor (section 4.1.2). The quantum yield of a dye can be defined as the ratio of the number of photons emitted to the number of photons absorbed, and gives an idea of the efficiency of the fluorescence process. The quantum yield of Cy5 is around 25% [96] where as that of Rhodamine 6G is around 95% [97]. The
quantum yield of weak fluorophores increases in the presence of metallic surfaces [8] and hence, we can expect the quantum yield of Cy5 to increase due to the decrease in lifetime [98]. However, even after the increase in the quantum yield of Cy5, the average enhancement from the nano-gaps is lower than in the case of R6G dye. This behavior might be due to some of the following reasons:

1. The quantum yield of Cy5, even though it increases due to the presence of a metal surface, will not be as high as 95%.

2. The Cy5 dye film is separated from the silver surface by a 30 nm titanium dioxide layer, where as the R6G dye film is right on top of the silver surface without a spacer layer. Therefore, the Rhodamine experiences quenching for the first 10 nm and the top 20 nm of the Rhodamine-doped film will experience the evanescent field of the gratings (refer to figure 4.19). The Cy5-doped film only experiences the evanescent field after the 30 nm separation distance and hence, will not experience the quenching since it is sufficiently farther than 10 nm from the silver surface.

Overall, the silver gratings fabricated by the novel fabrication technique employed here were able to produce an average enhancement of 26 times with respect to a microscope glass slide using epi-fluorescence microscopy. This compares very well the enhancement factor of 30 obtained for silver gratings fabricated by Cui et al using interference lithography and optimizing the grating height parameters [15]. However, through the introduction on nano-gaps in the gratings fabricated in this study, we can achieve enhancement factors of up to 68 times on average and a maximum
enhancement of 118. Kinkhabwala et al have shown large electromagnetic enhancements from gold bowtie nano-antennas, and single molecule fluorescence enhancements of up to 1340 [13], although they employ e-beam lithography.

4.2 Field distributions using FDTD

Although the applications of plasmonic nanostructures are important, a physical picture of the phenomena taking place at the nanoscale is just as important to be able to fabricate more efficient and realistic plasmonic features. In this case, it is important to realize the near-field electromagnetic distributions to compare the experimental results of fluorescence enhancement to electromagnetic field enhancement from the modeled simulations of the nano-structures. For this purpose, electromagnetic field computations were performed using commercially purchased software - FullWAVE (RSoft Inc.) based on the FDTD algorithm as described in section 2.4.

4.2.1 Silver gratings in 2D

The first configuration used for field simulations was a silver grating with a semi-circular shape, a grating pitch of $\Lambda = 400$ nm, a grating height of $h = 60$ nm, and surrounding dielectric as air (Figure 4.20). A rectangular launch field was described with unit power at $z = 0.35$ $\mu$m and the angle of incidence could be varied by the user. A monitor (Monitor #1 – green) was placed above the launch field at $z = 0.38$ $\mu$m, measuring the received power and averaging it over time to provide the final output. This monitor can be thought of as a detector that measures reflectance. The computational domain was defined from $x = -0.2$ $\mu$m to +0.2 $\mu$m and $z = 0$ $\mu$m to 0.4
μm. A periodic boundary condition was used in the x-direction since the grating is periodic in this direction. For the maximum z value, a PML boundary condition was used while the minimum z value used a PEC boundary condition as this boundary is inside the silver surface and can be regarded as a perfect electrical conductor. The grid size was uniform and equal in both directions and chosen to be Δx = Δz = 0.005 μm. The FDTD stop time was set to 40 μm (in units of cT) and a continuous wave (CW) excitation was chosen to be incident upon the silver structure.

Figure 4.20 (a) RSOFT CAD window display showing the semicircular grating structure created to perform the simulations for periodic silver gratings. (b) A contour map of the silver structure showing the value of the real part of the dielectric permittivity (ε_r) in the computational domain for 495 nm TM polarized light.
The reflected power was detected using the monitor and plotted as a function of the angle of incidence (varying from 0° to 18°; steps of 0.01°) for an excitation wavelength of 495 nm and TM polarized light (E-field in the x-z plane). Figure 4.21 shows the reflectance as a function of the angle of incidence for the above simulation parameters. It is clear that at an incidence of 8°, the reflectance drops to a minimum which is characteristic of the SPR dip as seen in silver gratings. Hence, this structure simulates the fabricated samples very closely as the silver plasmonic gratings also showed a θ_{SPR} = 8° for 495 nm TM polarized light in air (section 3.3).

![Figure 4.21](image)

**Figure 4.21** Reflected power as measured by the monitor as a function of the angle of incidence. The SPR angle is denoted my the sharp dip in the reflectance value at 8° for 495 TM polarized light. This angle denotes the optimal coupling for the incident photons to the surface plasmons on the silver grating surface.

After the configuration and the simulation parameters were established for the silver gratings in two dimensions, the reflectance was monitored for incident wavelength ranging from λ_{inc} = 400 nm to λ_{inc} = 600 nm (steps of 4 nm) and the incident angle from 0° to 40° (steps of 0.5°). This provides a dispersion relation for the SPR angle versus SPR wavelength for the silver grating (Figure 4.22), and is linear in nature as seen
from the experimentally measured reflectance on the fabricated silver gratings (section 3.3).

Figure 4.22 A color-coded map of the reflected intensity detected by monitor #1 for the visible wavelengths and the incidence angle ranging from 0° – 35° for TM-polarized light. The pink regions denote the minimum reflectance and denote the resonance angle for a particular wavelength. The dispersion shows a linear relation between wavelength and SPR angle in this region.

Similar curves can be obtained for different dielectrics surrounding the silver gratings, easily calculate using equation 2.15. Figure 4.23 below shows the SPR angles for different wavelengths in the visible range for various dielectrics.

Figure 4.23 The SPR angle as a function of the incident wavelength for the silver gratings calculated using the dispersion relation for different dielectric surroundings.

To map the near-field intensity distributions for the silver-air interface, another monitor (Monitor # 2 – light blue) was placed in the x-z plane which measured the
magnetic field going out of the plane (\(H_y\)) for the TM polarized 495 nm light. In figure 4.24, three different excitation angles are shown, and it is evident that the maximum field intensity (\(H_y^2\)) is seen at the \(\theta_{\text{SPR}} = 8^\circ\) as opposed to 0° or 18° incidence.

![Figure 4.24 Field intensity distributions \((H_y^2/H_{y0}^2)\) for three different angles of incidence with 495 nm TM polarized light. An angle of 8° provides the maximum field enhancement at the grating surface while moving away from the SPR angle leads to lesser enhancement and lower field concentration.]

The field intensity has also been plotted as a function of the distance into the dielectric (z) from the silver grating surface at x = -0.1 μm (Figure 4.25) and shows an evanescent profile for the SPR case as discussed in section 2.1. The maximum intensity is achieved at the silver-air interface and exponentially decreases as it moves into the air. The intensity reaches 1/e (37%) of its maximum value at a distance of \(1/k_z'' = 47\) nm away from the interface which is also known as the “decay length”.

![Figure 4.25 The field intensity as a function of the distance away from the metal-air interface, showing an exponential decay as expected for surface plasmon resonance and a \(k_z'' = 0.021\) nm\(^{-1}\).]

Based on the field intensity obtained above and the quenching behavior described in section 2.3 earlier, the optimal fluorophore placement can be chosen by
taking these two competing phenomena into account. Figure 4.26 shows the normalize
field intensity of the evanescent surface plasmon on the silver gratings, the emission
efficiency of a dye in the presence of a fluorophore (refer to equation 2.13), and the
product of both these curves. From this curve, one can see that the optimal fluorophore
to grating distance, $d_{opt}$, is 11 nm for a dye with förster radius, $d_F = 5$ nm.

![Figure 4.26](image)

**Figure 4.26** (a) Normalized SPR intensity at resonance and fluorophore emission intensity as a function of the distance between fluorophore and metal. The optimal separation is 11 nm for a förster radius of 5 nm, and the maximum efficiency also decreases with increasing $d_{opt}$. (b) The optimal distance for different förster radii.

The optimal distance also changes as a function of the förster radius and it can be seen that the maximum normalized intensity also decreases with increasing förster radius.

### 4.2.2 Nano-gap embedded Silver gratings in 3D

After establishing the correct grating structure that gives us the closest results to fabricated silver gratings, we used the same structure in three dimensions with the inclusion of the nano-gap to see the near-field profiles for a nano-gap embedded in silver gratings. Since the nano-gap is of finite length, we cannot use the periodic boundary conditions anymore and will have to use PML in the x-direction. The new structure created is shown in Figure 4.27 where the nano-gap with a lateral width of 20
nm cuts across a silver grating structure with 21 periods. The computational domain was defined from \( x = -4.2 \, \mu m \) to \( +4.2 \, \mu m \) (PML boundary condition at both boundaries), \( y = 0.03 \, \mu m \) (PEC) to \( 0.3 \, \mu m \) (PML), and \( z = -2.5 \, \mu m \) to \( +2.5 \, \mu m \) (PML at both boundaries). The grid size for this three-dimensional computational domain was selected to be \( \Delta x = \Delta y = \Delta z = 0.01 \, \mu m \). The FDTD stop time was set to 40 \( \mu m \) (in units of \( cT \)) and a continuous wave (CW) excitation was chosen to be incident upon this 3D nanostructure.

![Image](image_url)

**Figure 4.27** (a) RSOFT CAD window showing the grating structure in four different views. The structure comprises of a nano-gap (20 nm) intersecting a periodic silver grating with 21 periods in the x-z plane. (b) Contour profile of the nano-gap embedded in silver gratings in three different planes.

To choose the number of periods necessary to get a plasmonic interaction from the designed configuration, a convergence study was performed by varying the number
of periods, \( N_p \) from 1 to 29. Figure 4.28 shows how the reflectance curve changes for the different number of periods under a 495 nm TM polarized excitation in a three dimension computational domain. As \( N_p \) increases beyond 21, the reflectance curve moves towards the periodic boundary condition with the SPR angle being well-defined at 8 degrees. Hence, in the interest of keeping the computational domain smaller to avoid unrealistic simulation times, \( N_p = 21 \) was chosen to be the optimal number to see the surface plasmon resonance from the silver gratings.

![Figure 4.28](image.png)

**Figure 4.28** Reflectance spectra obtained for silver gratings with a grating pitch, \( \Lambda = 400 \) nm and a grating height of, \( h = 60 \) nm with different number of periods, \( N_p \), with \( N_p = \text{inf} \) denoting a periodic boundary condition.

Now, to see the field intensity distribution at the intersection of the nano-gap with the silver gratings, a spatial monitor was placed at \( y = 0.08 \) um. The excitation conditions were chosen as 495 nm TM polarized light for an incidence angle of \( 8^\circ \), to achieve surface plasmon resonance on the silver gratings. For the same optical launch
conditions, three different configurations (Figure 4.29) were compared: a nano-gap with the gratings, the gratings without a nano-gap, and a nano-gap on a flat film. For the same resonant excitation conditions the nano-gaps embedded in gratings show much larger near-field intensity when compared to the gratings and the nano-gap on a flat silver film. This proves that the field enhancement in this case is due to the combined effect of grating coupled surface plasmon resonance (GC-SPR) and localized surface plasmon resonance, whereas in the case of silver gratings without nano-gaps the field enhancement is only due to the GC-SPR and in the metallic nano-gap on a flat surface, the enhancement is entirely due to a localized SPR phenomenon.

![Figure 4.29](image)

**Figure 4.29** Field intensity distributions at the air-silver interface (top-view) for 495 nm TM polarized light (a) silver grating (b) silver nano-gap and (c) nano-gap embedded in silver grating.

The intensity values obtained from the monitor placed at \(y = 0.08\, \text{um}\), i.e., at the silver surface, for the different configurations in Figure 4.21 have been listed in Table 4.5 below. The intensity values listed are for the point \((0, 0, 0.08)\) in the field distribution maps.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat silver film</td>
<td>1</td>
</tr>
<tr>
<td>Nano-gap on flat silver</td>
<td>3</td>
</tr>
<tr>
<td>Silver grating</td>
<td>52</td>
</tr>
<tr>
<td>Nano-gap embedded in Silver grating</td>
<td>299</td>
</tr>
</tbody>
</table>

**Table 4-5** The field intensity values for the different substrates under same excitation conditions at the point \((0, 0, 0.08)\).
Therefore, from the FDTD simulations, it is evident that the nano-gaps embedded in gratings have much higher field intensities when compared to the gratings and the nano-gap on a flat silver film under resonance conditions. However, these field enhancements are much higher compared to the average EF of 68 times observed using fluorescence studies for the nano-gap embedded gratings and an average EF of 26 times for the silver gratings. This is because the fluorescence enhancement depends not only on the electric field enhancement due to the plasmonic structure resonance, but is also heavily dependent on the optical properties of the fluorophore as well as the optical filters used to measure the fluorescence intensity.

4.3 Surface Enhanced Raman Studies

Besides providing fluorescence enhancement due to the increased field intensity at the intersection of the nano-gap and gratings, the fabricated substrates are potential candidates for surface enhanced Raman scattering (SERS) studies [50, 99]. Raman spectroscopy is a widely used technique which provides a chemical fingerprint for probe molecules based on the presence of certain vibrational modes within the chemical bonds of the structure [100-102]. Raman spectroscopy uses a monochromatic light source to excite a molecule through an objective lens and detect the emitted photons as a result of inelastic scattering via a strong detector and a grating spectrometer. This inelastic scattering occurs due to the interaction with vibrational modes or phonons and can lead to a Stokes or Anti-Stokes Raman shift in the wavelength. These shifts provide us information about the vibrational modes of a molecule by showing intensity peaks at
certain wave numbers, thus providing a chemical fingerprint for a given molecule. The Raman intensity is much less than the fluorescence intensity and requires very high quality detectors. Surface enhanced Raman scattering (SERS) provides huge Raman signal enhancement through the use of plasmonic substrates for electromagnetic (EM) field concentration on the nanoscale. As in the case of fluorescence enhancement, the strong localization of light at the surface of the nano-gap embedded silver gratings, provides huge gains in the Raman signal, thereby improving the SNR and easy identification of probe molecules.

The enhancement of Raman signal in the case of SERS can be explained due to two contributing factors, i.e., the electromagnetic (EM) enhancement and the chemical (CM) enhancement. The EM enhancement is believed to be a result of the enhanced electromagnetic field due to the excitation of propagating or localized surface plasmons in the case of metal nanostructures. The theoretical electromagnetic enhancement factor (EF) is given by [103-105]:

$$EF = \left| \frac{E_{\text{out}}}{E_0} \right|^4$$

Where $E_{\text{out}}$ is the electric field amplitude at the metal surface under resonance and $E_0$ is the incident electric field amplitude.

The CM enhancement on the other hand is believed to arise as a result of charge transfer between a SERS substrate and the molecule being probed [106]. For example, graphene can provide significant chemical enhancement due to the ease of charge transfer between certain molecules and the $\pi-\pi$ stacking of graphene [107, 108].
The fabricated nano-gap embedded gratings were tested for their performance as SERS substrates using 2-napthalenethiol as the probe molecule [109, 110]. The silver plasmonic substrates were immersed in a solution of 1mM naphthalenethiol in toluene and left overnight to form a self-assembled monolayer (SAM). Thiols form excellent SAM layers on metal surfaces due to the strong affinity between the metal surface and the hydrophilic head group (-SH) [111]. After overnight incubation, the substrates were rinsed thoroughly with toluene and dried with nitrogen. Raman spectra for different substrates were acquired using a commercial Raman microscope (Renishaw inVia Reflex Raman microscope) which includes a 514 nm Argon laser line, an 1800 lines/mm grating for spectral resolution of the Raman scattered light, and a CCD detector. The spectra shown in Figure 4.30 were collected using a 20x objective, a laser power of 7.7 mW and detector integration time of 10 seconds.

Figure 4.30 Raman spectra for a self-assembled monolayer of 1mM 2-Naphthalenethiol obtained on different substrates including nano-gap embedded gratings and commercially available Klarite substrates. The spectra were obtained using a 514 nm Argon line with a 20x objective.

A comparison between various substrates – flat silver film, flat gold film, silver grating, and silver grating with nano-gaps, nano-gaps on a flat silver film, commercial gold Klarite substrate, and silicon is shown in Figure 4.30 and Figure 4.31.
Based on the spectra collected above, the intensities of two different peaks were compared for the different substrates. It is clear that the nano-gap embedded gratings provide much higher Raman intensities than flat silver films or nano-gaps on flat silver films (Figure 4.32). There is not a big difference between the gratings without any nano-gaps and the silver gratings with nano-gaps. This can be due to the fact that when the Raman spectra is collected, the spot size of the laser (10 μm) covers a large area of the grating and only a small portion of the nano-gap. So in this scenario, we can expect enhancements very similar or slightly better than the gratings (without nano-gaps) substrate.
Figure 4.32 SERS intensity for five different substrates for two vibrational modes of 2-napthalenethiol: 1066 cm⁻¹ and 1380 cm⁻¹.

The nano-gap embedded silver gratings can thus provide much better Raman signals as compare to flat metallic surfaces due to the EM enhancement provided by the localization in the near-field. A very important figure of merit for SERS applications is the SERS enhancement factor or G, which can be calculates as follows [112, 113]:

\[
G = \left( \frac{I_{\text{SERS}}}{N_{\text{SERS}}} \right) \left( \frac{N_{\text{RAMAN}}}{I_{\text{RAMAN}}} \right) = \left( \frac{N_{\text{RAMAN}}}{N_{\text{SERS}}} \right) \left( \frac{I_{\text{SERS}}}{I_{\text{RAMAN}}} \right)
\]

Where, \( I_{\text{SERS}} \) is the intensity of the particular peak of 2-napthalenthiol (NT) when measured on SERS substrates (enhanced signal) while \( I_{\text{RAMAN}} \) represents the intensity of same peak in un-enhanced Raman spectra measured in neat form. \( N_{\text{SERS}} \) is the number of NT molecule contributing to the SERS signal on the substrate excited by laser beam.
and $N_{\text{RAMAN}}$ is the number of molecules contributing to the un enhanced Raman signal. $N_{\text{SERS}}$ and $N_{\text{RAMAN}}$ can be calculated as follows [111]:

$$N_{\text{SERS}} = AR\mu$$  \hspace{1cm} (2)

$$N_{\text{RAMAN}} = AH\rho$$  \hspace{1cm} (3)

Hence, $G = \left( \frac{AH\rho}{AR\mu} \right) \left( \frac{I_{\text{SERS}}}{I_{\text{RAMAN}}} \right) = \left( \frac{H\rho}{R\mu} \right) \left( \frac{I_{\text{SERS}}}{I_{\text{RAMAN}}} \right)$  \hspace{1cm} (4)

Where $A$ is the illuminated area of the substrate that contributes to the SERS, while $R$ is the fraction of the surface area of substrate, which is illuminated. It is measure of the surface roughness factor of the substrate that contributes to the enhancement and $\mu$ is the packing density of NT molecules. $H$ is the apparent height of the NT layer contributing to the Raman signal [3] and $\rho$ is the density of NT molecules.

$\mu$ of NT was found to be $2.4 \times 10^{14}$ molecule/cm$^2$ [111], $H$ is on the order of 20 $\mu$m for a 50X objective lens [112] and $\rho$ to be $5.8 \times 10^{21}$ molecule/cm$^3$ (Sigma Aldrich).

![Graph showing Raman Shift vs Counts for different samples](image-url)
The spectra obtained above are the rough Raman spectra and the intensity of the 1380 cm\(^{-1}\) peak was analyzed after subtracting the baseline (Figure 4.33). Since the incident power is different for all the substrates and the neat Raman signal (powder form), the equation for G will change as follows:

\[
G = \left( \frac{H \rho}{R \mu} \right) \left( \frac{I_{SERS}}{P_{SERS}} / \frac{I_{RAMAN}}{P_{RAMAN}} \right)
\]

**Figure 4.33** Raman spectra of 2 naphthalenethiol SAM taken with a 50x objective, 10s integration time, 7.7 mW laser power on various substrates with a 514 nm Argon line. (b) Raman spectra of powdered NT taken with 50x objective, 10s integration time, 0.154 mW laser power.
After doing all the baseline subtractions and using the above formula, the value of $G$ was calculated for all the 4 different substrates using $R = 1$ at this point since the value of $R$ is not know.

<table>
<thead>
<tr>
<th></th>
<th>$I_{SERS}$</th>
<th>$I_{RAMAN}$</th>
<th>$G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG-Ag grat</td>
<td>5991</td>
<td>2837</td>
<td>2.041E+03</td>
</tr>
<tr>
<td>Ag grat</td>
<td>7518</td>
<td>2837</td>
<td>2.562E+03</td>
</tr>
<tr>
<td>Only NG</td>
<td>619</td>
<td>2837</td>
<td>2.109E+02</td>
</tr>
<tr>
<td>Flat Ag</td>
<td>406</td>
<td>2837</td>
<td>1.383E+02</td>
</tr>
</tbody>
</table>

*Table 4-6* Comparison of Raman enhancement factor $G$ using the formula described above for different substrates.

The value of $R$ will not be the same for all substrates since the surface area exposed will be different. Therefore, the value of $G$ will also be different for all the different substrates. Let us say $R = 0.001$, then the value of $G$ will be on the order of $10^6$ for the nano-gap embedded silver gratings.
4.4 Single molecule imaging

Since the advent of techniques such as Confocal Fluorescence Microscopy (CFM) [114], Near-field Scanning Optical Microscopy (NSOM) [115] and Total Internal Reflection Fluorescence Microscopy (TIRFM) [116], single molecule imaging has been made possible via optical means [93]. The ability to study molecules individually at the single molecule level can provide a plethora of information about the motion and interactions of these molecules within different environments. TIRFM setups utilize a high intensity laser along with very high NA oil-immersion objectives to produce an evanescent field which excites the dye molecules leading to fluorescence based detection of single molecules [117]. Usually, wide-field microscopes are unable to resolve single molecules due to high background signals and low signal to background ratio. However, placing the fluorophores in close proximity of the enhanced electric field of the nano-gap embedded plasmonic gratings can provide improved contrast and single molecule resolution [13]. Using the same unmodified epi-fluorescence setup as described before, we have been able to obtain single molecule fluorescence data by immobilizing 3 nm and 20 nm Rhodamine-doped organosilicate nanoparticles (DOSNP) on top of the nano-gap embedded silver gratings.

The silver nano-gap embedded gratings were prepared as described previously with a 9 nm layer of SiO$_2$ (measured using ellipsometry) on top deposited using e-beam evaporation. In addition to protecting the silver surface from oxidation, the silica layer also acts as a spacer layer that reduces the dye-metal quenching up to certain extent.
The silica surface was later deposited with a self assembled monolayer of poly,4-vinlyridine (PVP) which is described below:

**PVP treatment**

- 1% by weight solution of PVP in Isopropanol (IPA) was prepared. The substrates were then immersed in the solution poured in a beaker (previously cleaned with IPA) for 2 hours.
- Substrates were then rinsed thoroughly with IPA to remove the weakly bonded PVP from the substrates and dried with N₂.
- Substrates were baked on a hotplate at 120° C for 1 hour which cures the PVP film, evaporates all IPA solvent, and provides a more uniform and consistent film.

PVP was chosen as it is an excellent adhesive for nanoparticles due to the strong affinity of the pyridyl group to metals and the ability to form hydrogen bonds with polar species [118, 119]. After including both the silica as well as the PVP layers on top of the silver surface, the fluorophores will be at least 12 nm away from the quenching surface, which is very close to the optimal fluorophore position for a förster radius of 5 nm (Figure 4.19 (a)).

**Problems with imaging single particle fluorescence on silver substrates**

Performing single molecule studies with silver substrates is a big challenge due to the spontaneous formation of fluorescent silver clusters on silver surfaces in ambient conditions. These silver fluorescent clusters [120, 121] also show blinking characteristics.
that are similar to individual 3 nm DOSNP and hence possess a huge problem when imaging these nanoparticles on a silver surface. Figure 4.34 shows the presence of fluorescent blinking silver clusters on silver gratings as well as flat silver films. The fluorescence images were taken using the previously described epi-fluorescence setup with a FITC filter cube set and 400 ms exposure time of the CMOS detector.

Figure 4.34 (a) Fluorescence micrograph showing fluorescent silver clusters on silver grating substrates taken with a FITC filter cube set, 60x water objective, CMOS Gain of 200 and exposure time of 400ms. (b) Bright field image of the exact same area as in (a). (c) Fluorescence micrograph showing fluorescent silver clusters on flat silver films taken with a FITC filter cube set, 60x water objective, CMOS Gain of 200 and exposure time of 400ms.(d) Bright field image of the exact same area as in (d).

These substrates therefore cannot be used for imaging 3 nm DOSNP because of the interfering background fluorescence form the silver clusters under the optical conditions used above. However, using a different fluorescence filter set and reduced exposure time of the detector, we can eliminate the signal from the fluorescent silver
clusters. Moreover, the deposition of a dense yet thin layer of silica and a SAM layer of PVP will also prevent the formation of fluorescent Ag clusters up to a certain extent. Figure 4.35 shows the fluorescence micrograph and the bright-field image of the exact same area using the 60x objective and R6G filter set (specifications also shown in the same figure) for a nano-gap embedded grating covered with silica and PVP. The fluorescence image was taken with an internal CMOS gain of 255 and an exposure time of 100 ms. It is evident in this case that there is no additional blinking fluorescence from the silver clusters and hence, these substrates can be used for imaging other fluorescent nanoparticles. As the exposure time is increased for the same illumination conditions, the presence of silver clusters is more visible and hence might interfere with the signal from other fluorophores present on the surface. Therefore, 100 ms was chosen as the optimal exposure time to eliminate silver cluster fluorescence and image other fluorophores or fluorescent nanoparticles.
4.4.1 Imaging 3 nm DOSNP on Nano-gap embedded gratings

After optimizing both the substrate and optical conditions for eliminating the interference from fluorescent silver clusters, individual 3 nm DOSNP were studied. The PVP provides a 2-3 nm uniform thin film that acts as an adhesive for most nanoparticles. Following the PVP assembly on the nano-gap embedded silver gratings with a 9 nm silica layer, the substrates were incubated in a solution of 3 nm Rhodamine-doped Organosilicate nanoparticles (DOSNP) in de-ionized (DI) water (20 µg/ml) for three different incubation times – 18 hours, 3 hours, and 30 minutes. Following the incubation step with the nanoparticles, the substrates were rinsed thoroughly with DI water. The substrates are now dried with N₂ leaving behind immobilized 3 nm DOSNP on top of the nano-gap embedded gratings (Figure 4.36).
**Figure 4.36** Schematic representing the cross-sectional layout of the 3 nm DOSNP immobilized on top of the silver nano-gap embedded gratings with a silicon dioxide spacer layer and self-assembled monolayer of PVP.

AFM images were taken on the different substrates with different incubation times to assess the density of the immobilized 3 nm DOSNP on the surface. Figure 4.37 shows the particle density on top of the silver gratings with the 9 nm silica layer and PVP SAM layer with three different incubation times.
From the AFM images shown above, it is evident that the 3 nm particle density is much lower for the 30 minute incubation time as compared to 18 hours and three
hours. This is because the nanoparticles have lesser amount of time to bind to the PVP layer and hence, the effective particle density on the surface is much lesser for lower incubation times.

Fluorescence images were also obtained for the sample prepared using the incubation procedure mentioned above using the R6G filter cube, the 40x objective (NA = 0.60), exposure time of 100ms, and a CMOS camera gain of 255, and are shown in Figure 4.38.

![Fluorescence micrographs](image)

**Figure 4.38** Fluorescence micrographs obtained using a 40x objective, R6G filter and 100 ms exposure time for 3 nm DOSNP immobilized on the nano-gap embedded silver gratings for three different incubation times (a) 18 hours (b) 3 hours and (c) 30 minutes.

The fluorescence images obtained above show that the fluorescence obtained is still pretty uniform over the plasmonic substrate surface and that the particle density on the surface needs to be reduced further. Therefore, the same incubation procedure was performed for 30 minutes but with a concentration of 0.2 µg/ml DOSNP in DI water, which ensured lower nanoparticle density on the substrates after immobilization. The AFM surface image for this sample (Figure 4.39) showed very low particle density as compared to the previous higher concentration of 20 µg/ml.
Fluorescence measurements of the nano-gap embedded gratings with a 0.2 µg/ml 3 nm DOSNP concentration (30 minutes incubation time) were performed using the same epi-fluorescence setup with a 60x water-immersion objective, R6G filter set, CMOS camera gain of 255 and an exposure time of 100ms. Fluorescence data was acquired in the form of videos and stored as an AVI file. Figure 4.40 shows individual frames extracted from the video file at time t = 0 seconds and t= 8.7 seconds. The second frame shows the presence of an individual nanoparticle which was not present in the first frame indicating the fluorescence blinking behavior of the 3 nm DOSNP.

Figure 4.40 (a) Fluorescence micrograph at t = 0 for the 3 nm DOSNP immobilized on the silver substrates (b) Fluorescence micrograph at t= 8.7 second for the 3 nm DOSNP immobilized on the silver substrates (c) Bright field image of the same area.
Since the signal to noise ratio is still very low, more extensive image processing and analysis needs to be done to further study the time characteristics of the 3 nm DOSNP.

4.4.2 Imaging 20 nm DOSNP on Nano-gap embedded gratings

A very similar study was performed by immobilizing 20 nm DOSNP on top of the nano-gap embedded silver gratings. The first study was performed without the presence of the silica layer, while the PVP SAM was still formed on top. The substrates were incubated in a solution of 20 nm Rhodamine-doped organosilicate nanoparticles (DOSNP) in de-ionized (DI) water (30 µg/ml) for 24 hours. Following the incubation step with the nanoparticles, the substrates were rinsed thoroughly with DI water. The substrates were then dried with N₂ leaving behind immobilized 3 nm DOSNP on top of the nano-gap embedded gratings. AFM images were obtained for the substrate prepared above at different steps of the procedure to show the change in topography after the immobilization of the 20 nm DOSNP on the silver surface with the PVP SAM layer (Figure 4.41).
Figure 4.41 The topography, amplitude and phase images for (a) silver gratings, (b) silver gratings with PVP SAM and (c) silver gratings with PVP SAM and 20 nm DOSNP immobilized for an incubation time for 24 hours and a concentration of 30 μg/ml in DI water.

Fluorescence measurements of the nano-gap embedded gratings with a 30 μg/ml 20 nm DOSNP concentration (24 hours incubation time) were performed using the same epi-fluorescence setup with a 60x water-immersion objective, R6G filter set, CMOS camera gain of 255 and an exposure time of 100ms. Fluorescence data was acquired in the form of videos and stored as an AVI file for a total of 3000 frames at 10 frames per second. Individual nanoparticle blinking was observed in this case arising from the nano-gaps embedded in the silver gratings and the blinking characteristics were analyzed by plotting the time traces of the blinking particles for one 20 nm nanoparticle as shown in Figure 4.42 [122]. The time traces correspond to individual pixels that correspond to the region of (a) nanoparticle fluorescence (b) background.
fluorescence of nano-gap and (c) background fluorescence of grating region in Figure 4.42.

![Graph](image.png)

**Figure 4.42** (a) Time traces of a single 20 nm DOSNP obtained from the sample prepared above showing intensity as a function of time (b) the background intensity from the nano-gap region where no blinking behavior is observed and (c) the background intensity from the grating region where no blinking fluorescence is observed from the 20 nm DOSNP.

The blinking characteristics of the individual 20 nm DOSNP show the existence of multiple fluorescence states and further analysis needs to be done to obtain statistically significant data. The individual time traces shown above prove the fact single nanoparticle fluorescence imaging can be possible using an epi-fluorescence setup and the fabricated nanogap embedded silver gratings.
5. CONCLUSION

In this study, a novel fabrication procedure has been described that enables cost-effective, fast, and easy production of nanoscale sub-wavelength plasmonic gratings. A store-bought HD-DVD was used as a master mold to replicate the pattern on to a PDMS stamp. PDMS micro-contact printing was used to stamp gratings made of PMSSQ polymer on glass and silicon substrates. The stamping procedure also introduced defects in the form of nano-gaps on the printed PMSSQ gratings as a result of tensile strain in the elastomeric PDMS stamp. After depositing silver on top of these nano-gap embedded gratings, the silver substrates were characterized using reflectance measurements and surface characterization was also performed using AFM and SEM. The reflectance setup helps in identifying the optimal wavelength and angle conditions for surface plasmon resonance.

The nano-gap embedded silver gratings provide huge near-field enhancements owing to a combination of localized surface plasmons at the nano-gap as well as propagating surface plasmons from the silver grating structure. This near-field concentration of electromagnetic energy can be used to excite fluorophores in the immediate vicinity. Fluorescence studies were performed with a thin Rhodamine-doped film deposited on top of the silver plasmonic substrates and fluorescence image analysis revealed an average enhancement of 69 times and maximum enhancement of 118 times for the nano-gaps in the gratings with respect to a microscope glass slide. Similar
results were obtained with a Cy5 dye and a titanium dioxide layer on top of the silver nano-gap embedded gratings.

FDTD simulations were also performed to see the electric field distributions at the nano-scale and to observe the response of the plasmonic structures with different excitation conditions. The simulation results match well with the experimental observations showing much higher field concentrations in the nano-gap embedded gratings than just gratings or just nano-gaps on a flat silver film. Raman studies were also performed on a self assembled monolayer of naphthalenethiol, showing the potential of the fabricated plasmonic gratings as SERS substrates. Finally, the huge filed enhancement was used to image individual 3 nm and 20 nm Rhodamine-doped nanoparticles using an unmodified epi-fluorescence setup and a 60x water-immersion objective. These nanoparticles can be easily tagged to antibodies and proteins, enabling single molecule imaging.
References


