

Super-Resolution Imaging of Plasmonic Hotspots

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Abstract

As the need to control and manipulate light on the nanoscale increases, intense expectations are being placed on the field of plasmonics to provide novel and consistent ways of controlling light, to achieve the desired optical properties and performance on these scales for both scientific and industrial applications. These properties include, high electromagnetic near-field enhancements, designer optical surfaces with control over scattering and absorption properties and nanoscale light waveguiding. One of the promising properties of plasmonics is their ability to generate electromagnetic (EM) hotspots. These are near-field structures, small in volume, but with extremely high field intensities. To better understand and apply these phenomena, techniques to characterize and image the spatial structure of these fields will become important. This is, in part, due to the extreme sensitivity of these fields to small structural and environmental changes to the plasmonic antenna.

Here we present work on a single molecule fluorescence localization based super-resolution technique for the study and imaging of electromagnetic near-field structures generated around plasmonic antennas, with a special interest in the study of EM hotspots. The experimental work encompasses developing a suitable optical setup for fluorescent molecule detection, sample design and fabrication using electron beam lithography, as well as finite difference time domain simulations of plasmonic antenna properties. The technique has been used to probe the near-fields around a variety of plasmonic antennas.

Analysis of the results demonstrated that the localization process can be complicated by the coupling of the fluorescence emission with the plasmonic system. When emission-coupled events occur, the results generally do not report the real position of the molecules, nor the EM enhancement distribution at the illuminating wavelength. To circumvent this issue, fluorescent molecules with a large Stokes shift have been used in order to spectrally decouple the emission process of the dye from the plasmonic system, leaving only the absorption strongly in resonance with the enhanced EM field in the antenna's vicinity. The real position of the emitters in this complex but interesting scenario are then found directly. The technique is demonstrated to provide an effective way of exploring either the EM field or the LDOS with nanometre spatial resolution.

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Publication

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Acronyms

AFM	Atomic Force Microscopy
APD	Avalanche Photo Diode
CCD	Charge-Coupled Device
CL	Cathode Luminescence
CW	Continuous Wave
DF	Dark Field
DI	Deionised
DMSO	Dimethyl Sulfoxide
EELS	Electron Energy Loss Spectroscopy
EM	Electromagnetic
FDTD	Finite Difference Time Domain
FWHM	Full Width Half Maximum
IPA	Isopropyl Alcohol
LDL-R	Low Density Lipoprotein Receptor
LDOS	Local Density Of States
LSR	Least Squared Regression
MIBK	Methyl Isobutyl ketone
MLEM	Maximum Likelihood Estimation Method
NA	Numerical Aperture.
NP	Nanoparticle
PAINT	Point Accumulation Imaging in Nanoscale Technique
PALM/ fPALM	Photoactivated Localization Microscopy/ Fluorescent Photoactivated Localization Microscopy

PB/PO	Pacific Blue/ Pacific Orange
PID	Proportional Integral Differential
PL	Photoluminescence
PMMA	Poly(methyl methacrylate)
PSF	Point Spread Function
PZC	Potential of Zero Charge
QE	Quantum Efficiency
QY	Quantum Yield
SAM	Self-Assembled Monolayer
SCD	Surface Charge Density
SEM	Scanning Electron Microscopy
SERS	Surface Enhanced Raman Spectroscopy
SIM	Structured Illumination Microscopy
SNOM	Scanning Near-field Optical Microscopy
SPC	Single Photon Counter
SPP	Surface Plasmon Polariton
SR	Super-Resolution
STED	Simulated Emission Depletion Microscopy
STORM	Stochastic Optical Reconstruction Microscopy
TE/TM	Transverse Electric/Transverse Magnetic
TIR	Total Internal Reflection

1. Introduction

1.1 Background and Motivation

Over last 40 years, swift development of new fabrication techniques has allowed for the control of visible light at a sub wavelength scales. Plasmonics is the study of these nanoscale light matter interactions for metallic structures and their applications [1-3].

The applications of plasmonics range broadly and plasmonic structures can find use in most areas of physics where light manipulation is needed. Plasmonics also offer extreme EM field intensities, due to its ability to reduce the mode volume of propagating or standing light waves, giving rise to high enhancements of many light matter interaction effects such as Raman scattering [4, 5], fluorescence [6], photochemical effects [7-9] and photothermal effects [10-12]. Another potential benefit of the reduced mode volume is the possibility of miniaturising optical components [13] down to similar scales which have been achieved by electronic structures.

The properties and potential applications of plasmonic devices have led to their use and the advancement of many areas of science including, sensing [14, 15], energy conversion [16, 17] and biomedical treatments [12, 18]

Many of the applications of plasmonics would benefit from knowledge of the near-field distribution around the plasmonic device. This information is normally only accessible via finite difference time domain (FDTD) simulations, which solve Maxwell's equations for a modelled optical structure, or through expensive and complex electron microscopy techniques [19]. It is experimentally complex to get a physical confirmation of the simulations, as advanced fabrication methods have driven down the size of structures to the nanoscale regime, which in turn produce extremely small field structures, which cannot be resolved by conventional optical microscopy. Conventional optical microscopy is limited by the diffraction nature of light giving rise to a lower resolution limit, which, for an optical system using visible light, is $> 200\text{nm}$. To be able map these minute sized plasmonic fields optically, a super-resolution (SR) approach is required. SR

approaches use some extra, already known information about the system being imaged, to bypass the diffraction limit and access higher resolutions.

In the area of biological imaging, SR fluorescent microscopy has been hugely successful and influential. So influential, in fact, that in 2014 the Nobel prize in chemistry was given to Erik Betzig, Stefan W. Hell and W. E. Moerner for their work developing SR. One SR technique [20] uses fluorescent molecules as spatial markers, conventionally attached to proteins of interest on a biological sample. By employing the molecules temporary dark states to temporally separate their emission, images of individual single molecules can be taken in a sequence. When the correct treatment for these far-field images is applied, it is possible to locate/localize the position of these molecules down to the single nm scale, way below the conventional diffraction limit of light. The positions of thousands of separate fluorescent molecules can then be localized and displayed together, giving a high resolution image of the tagged proteins.

By applying SR fluorescence microscopy ideas to plasmonic systems we can probe the near-fields generated around them. To map these near-fields, it was necessary to address the problem of coupling of emitted fluorescent light, from molecular probes, to the optical modes of the plasmonic system which can cause mis-localization of the fluorescent molecule's position [21-23]. We do this by spectrally decoupling the emission of the probes to reduce the mis-localization effect and increasing the precision of localization improving the EM field mapping. It was then possible to probe the near-fields with confidence and explore the effects of illumination polarization and structure variation on the near-field distributions.

We hope for this to fuel new work and understanding in the manipulation of light on these extreme scales.

1.2 Objectives and Goals

Here we document the major goals of this project followed by objectives that would need to be met to achieve these goals.

- i. To design and implement a functioning localization based super-resolution microscope for probing the near-field of plasmonic structures.
 - Achieve Total Internal Reflection (TIR) illumination
 - Collect TIR optically excited fluorescence
 - Collect single molecule fluorescence
 - Localize single molecule positions from far-field data using the maximum likelihood estimation method
 - Design and fabricate a suitable test plasmonic antenna
 - Demonstrate fluorescence enhancement for bulk fluorescence
 - Achieve Point Accumulation for Imaging in Nanoscale Topography (PAINT) like stochastic absorption to a sample surface demonstrating enhanced single molecule fluorescence leading to bright temporally spaced bursts of fluorescence
 - Use the technique to investigate hotspots generated in rough metallic films
 - Use the technique to investigate hotspots generated by a basic antenna
- ii. Probe the near-field of polarization dependent electromagnetic field geometries demonstrating change using localization based super-resolution microscope
 - Design and spectrally simulate suitable plasmonic structures
 - Produce simulations of polarization dependent electromagnetic field distributions
 - Fabricate and characterize sample geometries
 - Perform single molecule localization mapping of electromagnetic field and compare to simulation
- iii. Attempt to improve upon current single molecule super-resolution localization based techniques when applied to plasmonic structures.

1.3 Thesis Overview

This thesis begins with an introduction to plasmonics in Chapter 2, covering surface plasmon polaritons and localized surface plasmon resonances to enable a better understanding of plasmonics and EM hotspots.

Chapter 3 discusses fluorescence, covering plasmon enhanced fluorescence and single molecule fluorescence. Chapter 4 gives an overview of super-resolution microscopy, covering optical techniques, localization microscopy, applications of the techniques for probing plasmonic near-fields and an analysis of related and alternative imaging techniques that can be applied to the study of plasmonics systems.

The modelling and simulation of plasmonic structures using finite difference time domain simulations are discussed in Chapter 5. Details of simulations specific to this body of work are presented and discussed.

Experimental details are presented in Chapter 6, beginning with a description of the optical setup of the SR microscope. Sample fabrication, handling and characterisation details are presented. Details of fluorescent dyes suitable for the SR mapping done in this work are then given. The chapter concludes by considering the implementation of a sample tracking and stabilization technique for long sequential imaging.

Having discussed the relevant background and experimental details, initial results for PAINT based localization microscopy applied to various plasmonic samples are then presented in Chapter 7, while the limits and breakdown in the initial application of the technique are discussed in Chapter 8 considering current literature on the subject. A possible solution of spectral decoupling of the fluorescent emission is suggested.

Chapter 9 presents results from the application of this spectral decoupling. The results are numerically verified and the suitability of Stokes shifted PAINT microscopy for the study of plasmonic antenna is discussed.

Finally, conclusions of the work are presented followed by the consideration of possible improvements to the experiment and applications of the technique as areas of future work.

2. Plasmonics

2.1 An Overview of Plasmonics

Plasmonics is the study of the interaction between light and matter on the nanoscale. In a metal, neighbouring atoms are held together by metallic bonding via the sharing of electrons. This forms a freely moving, delocalised “sea” of electrons around a lattice of stationary positive ions. It’s these same free electrons that make metals good thermal and electrical conductors. When looking closely at this system, it is possible to think of the metal’s electron distribution acting similarly to a plasma, the electron sea is able to oscillate in response to outside influences. Within the bulk of a material the quanta of these oscillations are called plasmons.

When light interacts with these plasmon oscillations and are confined to the interface between a metal and dielectric, the photons and electron density oscillations form quasiparticles which are known as Surface Plasmon Polaritons (SPP). If we move from a continuous surface, like a metallic film, to a shaped material, such as a nanoparticle (NP), these SPPs can form standing waves much like that of sound waves on a length of string. These SPP standing waves are known as Localised Surface Plasmon Resonances (LSPR).

Historically plasmonic effects have been utilised since as early as 1500 BCE [24] to create vivid coloured glass by introducing metallic salts into the fabrication process. Probably the most famous example of plasmonic effects in old glass work is the Roman Lycurgus cup, which appears green when viewed in reflection or scattering but red when viewed in transmission.

At the beginning of the 20th century R. W. Wood published two papers which reported on observations of unusual interactions between visible light and a metallic grating [25, 26]. In the first of the two papers Wood reports on a new diffraction grating whose spectra could not be explained by the theory of diffraction gratings at the time. It would not be until the late 50s that these were explained by R. H. Ritchie as features of SPP on the metal gratings [27].

2.2 Surface Plasmon Polaritons

When trying to understand the nature and properties of SPPs it can be helpful to consider the dispersion relationship for a SPP propagating at the interface between a metal and a dielectric slab, as shown in Figure 2.1. Full derivations can be found in a review text such as [1]. The slabs are made up of a metal in the $z < 0$ region that is described via a dielectric function $\varepsilon_1(\omega)$, where $\text{Re}[\varepsilon_1] < 0$, which is a requirement of metallic properties and a dielectric slab in the $z > 0$ region with a positive real dielectric constant of ε_2 .

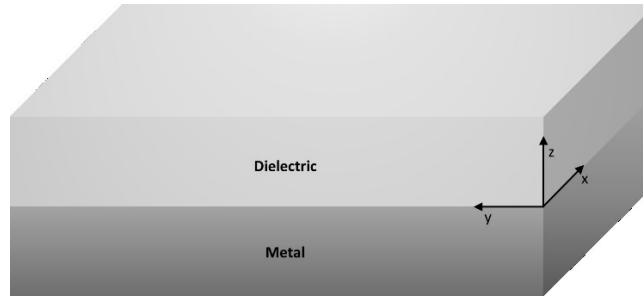


Figure 2.1 - **Dielectric/Metal interface.** A two layer metal dielectric setup for a SPP propagating along the x axis. [1]

To begin with we consider the transverse magnetic (TM) mode solution equations:

$$\text{For } z < 0 \quad H_y(z) = A_1 e^{i\beta x} e^{k_1 z} \quad (2.1)$$

$$E_x(z) = -\frac{iA_1 k_1}{\omega \varepsilon_0 \varepsilon_1} e^{i\beta x} e^{k_1 z} \quad (2.2)$$

$$E_z(z) = -\frac{A_1 \beta}{\omega \varepsilon_0 \varepsilon_1} e^{i\beta x} e^{k_1 z} \quad (2.3)$$

$$\text{For } z > 0 \quad H_y(z) = A_2 e^{i\beta x} e^{-k_2 z} \quad (2.4)$$

$$E_x(z) = \frac{iA_2 k_2}{\omega \varepsilon_0 \varepsilon_2} e^{i\beta x} e^{-k_2 z} \quad (2.5)$$

$$E_z(z) = -\frac{A_2 \beta}{\omega \varepsilon_0 \varepsilon_2} e^{i\beta x} e^{-k_2 z} \quad (2.6)$$

Here β is the propagation constant for the wave traveling in the x direction, k_i where $i = 1$ or 2 is the wave vector for each medium that defines the extension of the

evanescent field into each medium perpendicularly from the interface and A_i is a variable determined by the boundary conditions.

Each equation is broken into two main components; a traveling wave exponent $e^{i\beta x}$ describes a traveling wave in the x direction, controlled by the propagation constant, and the evanescent exponent $e^{k_1 z}$ and $e^{-k_2 z}$ which control the decaying evanescent properties of the fields away from the interface, with increasing or decreasing z . The decaying nature of the evanescent field results in mode confinement along the interface.

With proper consideration of the interface and satisfying the wave equation for a TM mode we can write the dispersion relation for an SPP propagating at the interface as:

$$\beta = k_0 \sqrt{\frac{\epsilon_1 \epsilon_2}{\epsilon_1 + \epsilon_2}} \quad (2.7)$$

Some of the properties of the surface charge interaction with the EM field are of significant importance. The first of these is that the momentum of a SPP mode is greater than that of a free space mode of the same frequency.

Figure 2.2 which is replicated with permission from [28], is an example of the momentum mismatch for SSP on metal dielectric interface to that of free space light at the same frequency. In

Figure 2.2, at frequency ω , the SSP momentum $k_{sp} > k_0$ the free space photon's momentum. This means that to excite a SPP the momentum between free space and SPP must be matched using one of a set of techniques including prism coupling or grating coupling [29, 30]. Nanoantennas can also be used to couple far-field light into the near-field and into a SPP.

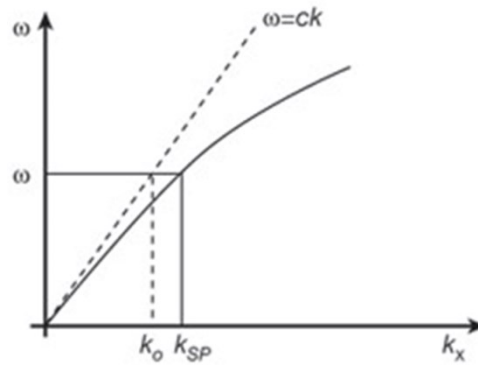


Figure 2.2 - **Graph showing dispersion curve for metal dielectric interface surface plasmon Polariton** (solid line) plotted with light line (dashed line) showing momentum mismatch. Replicated with permission from [28], Nature 2003.

Secondly, the real part of the dielectric functions of each medium at the interface must have opposite signs to support a plasmon mode to satisfy equations (2.1)-(2.6) to generate confined fields.

Thirdly, the field perpendicular to the interface decays exponentially with distance from the surface, leading to reduced mode volume size for the propagating wave when compared to its free space counterpart. This reduced mode volume ‘squeezes’ the light, increasing the field density giving higher field strengths than would commonly be available with the illumination used. Once generated, these SPP lose energy into the metal via electron electron scattering and phonon generation. This can give the SPPs short propagation distances when compared to purely optical modes and is one of the difficulties faced in the miniaturisation of optical components using plasmonics.

The nature of SPPs means they are well suited to the applications of sub wavelength optics and light manipulation on small scales. The field enhancements due to mode compression also make them ideal for countless applications including high harmonic generation [31] and light focusing [32].

2.3 Localised Surface Plasmon Resonances

While SPPs propagate along a metal dielectric interface, LSPRs are stationary and can be generated across metallic nanostructures as standing wave interferences between the free electrons in the metal and the light coupling to the metal. These interactions can massively change the properties that a material has in its bulk form. This change can be

controlled via the shape and size of the structures fabricated. Where gold is yellow in bulk, it can appear red through to violet when in a colloidal form, for sizes 5-90nm. These properties are the result of a resonant interaction between certain wavelengths of light with certain structures. To understand this resonant behaviour, we can look at a small sub-wavelength particle shown in Figure 2.3, located at the origin, subject to an electric field $\mathbf{E}$. For a sub-wavelength size sphere where $a \ll \lambda$ we can use a quasi-static approximation as, for a harmonically oscillating electric field, the phase over the small particle will be approximately constant. Therefore the static electric field $\mathbf{E} = E_0 \hat{\mathbf{x}}$ will be used.

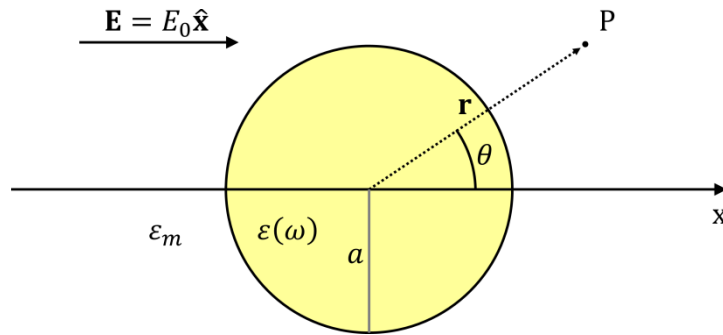


Figure 2.3 – **Sub-wavelength spherical particle in electrostatic field.** The sphere is homogeneous with dielectric function $\varepsilon(\omega)$, radius a and in isotropic, non-absorbing medium with a dielectric constant ε_m . Vector $\mathbf{r}$ is the position vector of point P which makes an angle of θ with the x -axis.

The equations for the potential Φ inside and outside the sphere are derived in [1] and can be written as:

$$\Phi_{\text{in}} = -\frac{3\varepsilon_m}{\varepsilon + 2\varepsilon_m} E_0 r \cos\theta \quad (2.8)$$

$$\Phi_{\text{out}} = -E_0 r \cos\theta + \frac{\varepsilon - \varepsilon_m}{\varepsilon + 2\varepsilon_m} E_0 a^3 \frac{\cos\theta}{r^2} \quad (2.9)$$

If equation (2.9) is examined, it is possible to see that it is made up of the electrostatic field applied at point P in the first term of the equation and the field produced by a dipole located at the origin in the centre of the particle as the second term of the equation.

$$\Phi_{\text{out}} = \Phi_{\text{Applied}} + \Phi_{\text{dipole}} \quad (2.10)$$

The dipole potential in equation (2.10) can be written as:

$$\Phi_{\text{dipole}} = \frac{\mathbf{p} \cdot \mathbf{r}}{4\pi\epsilon_0\epsilon_m r^3} \quad (2.11)$$

Where $\mathbf{p}$ is the dipole moment generated at the origin. By equating this dipole moment and the second term of equation (2.9) then rearranging it is found that the dipole moment for this system can be written as:

$$\mathbf{p} = 4\pi\epsilon_0\epsilon_m a^3 \frac{\epsilon - \epsilon_m}{\epsilon + 2\epsilon_m} \mathbf{E}_0 \quad (2.12)$$

Using equation (2.12) we can adjust equation (2.9) to be:

$$\Phi_{\text{out}} = -E_0 r \cos\theta + \frac{\mathbf{p} \cdot \mathbf{r}}{4\pi\epsilon_0\epsilon_m r^3} \quad (2.13)$$

It is helpful now to define a polarizability α where $\mathbf{p} = \epsilon_0\epsilon_m\alpha\mathbf{E}_0$

$$\alpha = 4\pi a^3 \frac{\epsilon - \epsilon_m}{\epsilon + 2\epsilon_m} \quad (2.14)$$

The polarizability is a measure of how easily a field $\mathbf{E}_0$ induces a dipole moment inside the sphere. From equation (2.14) it is possible to see that as ϵ (which is a function of wavelength) approaches $-2\epsilon_m$ the denominator approaches a minimum and α can become large. This is the Fröhlich criterion and means that a small change in field $\mathbf{E}_0$ can lead to a large induced dipole moment. This is resonance behaviour and at these resonant wavelengths the induced fields can be very large.

Antenna size, geometry and material composition can have a large effect on the behaviour of a LSPR. The size effect is demonstrated in Figure 2.4 which shows Dark Field (DF) microscopy images of two different lengths of aluminium rods. Figure 2.4 a) shows a schematic of the rod geometry used. In both case the width of the rods is 50nm and the height is 30nm. The samples were fabricated using e-beam lithography onto a glass substrate and the aluminium was deposited using evaporation deposition. Fabrication is discussed in more detail in section 6.2 Sample Setup and Fabrication.

In DF microscopy the sample is illuminated at with a white light illumination beam and light that is scattered way from this beam at a large angle is collected. This technique is highly suited for looking at highly scattering objects, like nanoantennas, giving high contrast. The DF setup was used in a vertical geometry using a tungsten source and a colour charge coupled device (CCD) camera was used to capture the images. For more details on DF microscopy and spectroscopy see section 6.3

The antennas in the array shown in Figure 2.4 b) are 100nm in length and the scattered light from the antenna is predominantly green, this is reflective of the resonant frequency of the LSPR generated for these antenna. In contrast Figure 2.4 c) which shows the scattered light, collected for length 150nm aluminium rods, is now yellow/orange. This indicates that the LSPR of the longer aluminium antenna has been red shifted with the increase in length. This ability to tune the resonant frequency of plasmonic antenna with geometry allows the selective engineering of the optical response of the antenna.

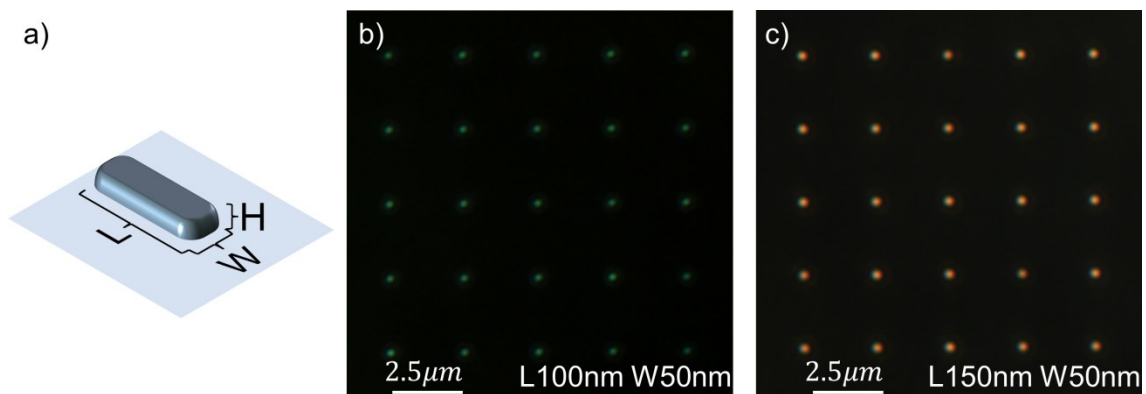


Figure 2.4 - **Dark Field microscopy of white light illuminated aluminium rod antenna.** a) schematic of individual aluminium antennas fabricated onto a glass substrate. The width W of each antenna is 50nm and the height H is 30nm. b) DF image of array of 100nm long aluminium rods. c) DF image of array of 150nm long of aluminium rods.

Antennas can also be designed to work in arrays, where each antenna interacts with its neighbours and can allow for other methods of light manipulation. This is demonstrated in Figure 2.5 which shows DF microscopy images of large arrays of closely spaced gold disks shown schematically in Figure 2.5 d) where D is the diameter of the disks and P the horizontal and vertical pitch of the disks. The DF images of arrays shown in Figure 2.5 a) b) c) are of 100nm diameter disks with a variation of pitch 400nm, 300nm and 200nm,

respectively. At the wide 400nm pitch in a) the scattered light collected is red, as the pitch is decrease to 300nm in b) the scattered like becomes more blue shifted and when the pitch is reduced further to 200nm the surface begins to act like a gold film and looks gold. Variations in disk diameter can also affect the scatted light Figure 2.5 b) e) f) shows arrays of a fixed pitch 300nm with disk diameters 100nm, 150nm and 200nm respectively. As the disk size increase the amount of scattered light collected increases, however, conversely to the size variation effect shown in Figure 2.4 no red shifting is noted with the increase in disk size. This is a good example of how the properties of an individual antenna can be changed by fabricating them in to coupled systems of antennas. The modes generated by the interaction of the disk arrays would give bright reflective measurements however they give dim scattering measurements from the centre of the arrays. However this brightness can be seen at the edges of the arrays where the mode fails as there is no longer neighbouring particles to cancel out scattering leading to the bright array edges seen in the DF images.

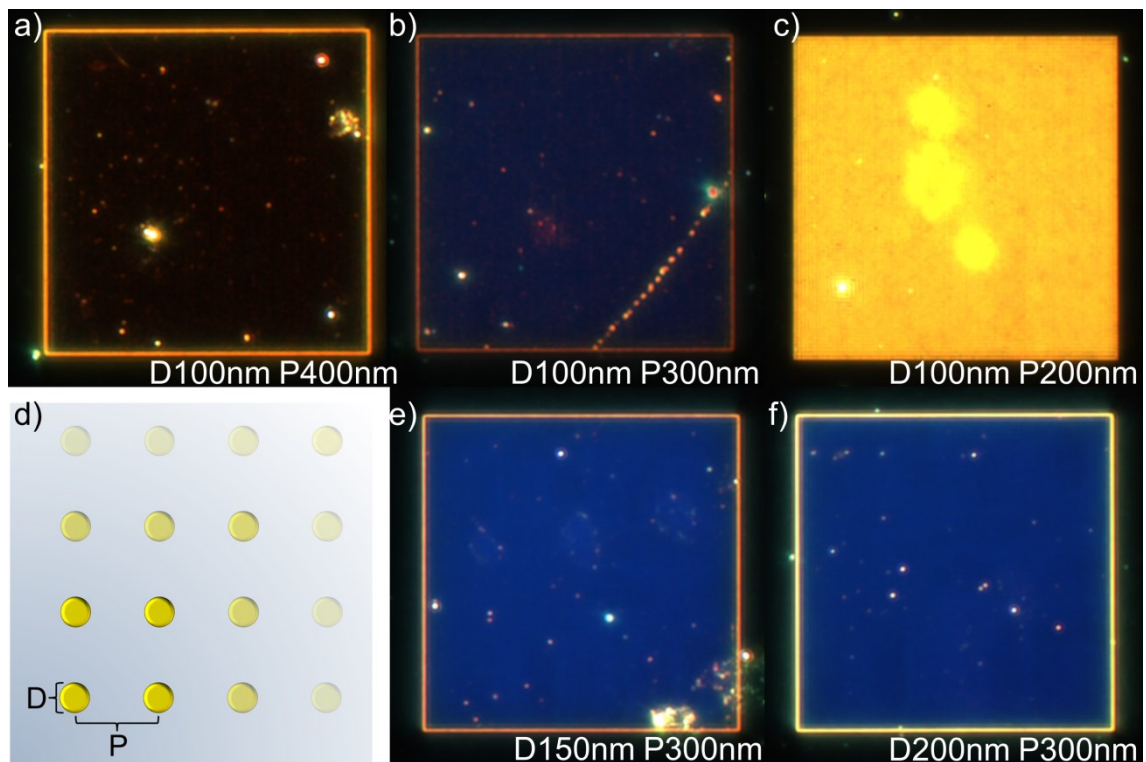


Figure 2.5 - Dark Field microscopy of white light illuminated large gold disk arrays. Arrays $20 \times 20 \mu\text{m}$ in size a) Array of 100nm diameter gold disks spaced by 400nm. b) Array of 100nm diameter gold disks spaced by 300nm. c) Array of 100nm diameter gold disks spaced by 200nm. d) schematic of gold disk array. e) Array of 150nm diameter gold disks spaced by 300nm. f) Large array of 200nm diameter gold disk spaced by 300nm.

This kind of optical response engineering has already found many applications, such as Surface-Enhanced Raman Scattering (SERS) spectroscopy [33]. LSPR in general have found applications in biological and chemical sensing. Of more importance to this work, due to the enhanced EM fields in the vicinity of LSPR they are used to enhance and manipulate the fluorescence of material in its vicinity.

3. Fluorescence

3.1 Introduction to Fluorescence

Fluorescence refers to the emission of light from a substance due to the relaxation of an electron which was excited via the absorption of an incident photon. Because of the singlet nature of the electronic states involved, the relaxation time or lifetime (τ) of a fluorescent system is very short (of the order 10ns). Fluorescent systems absorb light at a short wavelength and reemit the light at a longer wavelength, this is to say that the illumination photon has more energy than the emitted one, where the difference in energy between the two photons is lost to the internal conversion of the system. Fluorescence is an extremely common phenomenon of everyday life, with fluorescent materials added to household clothing detergent, fluorescent highlighters and even white paper often to make the subject appear brighter. In the last 30 years the use of fluorescence as a scientific tool has increased dramatically. Moreover, in the last 10 years it is spurred a revolution in super-resolution microscopy of biological structure. Before approaching these topics, a deeper understanding of physics of fluorescence is needed. Fluorescent systems are often described using a Jablonski diagram:

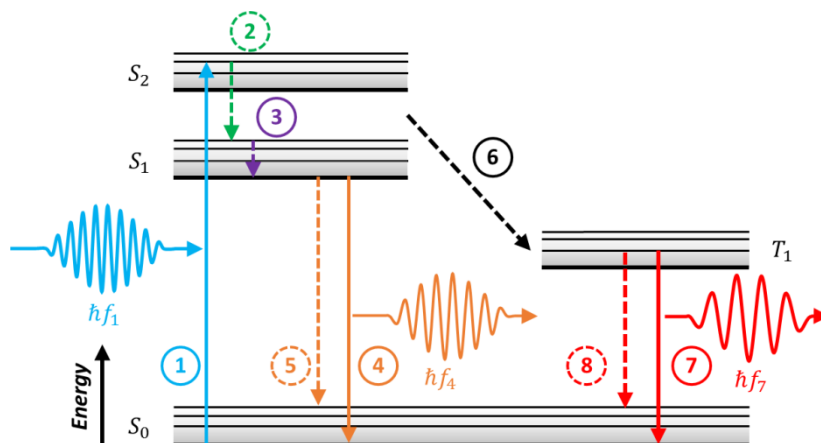


Figure 3.1 - **A Jablonski energy level diagram** of some of the possible absorption, emission and transition pathways in an example fluorescent system. Transition 1 is the absorption of an incidence illumination photon of frequency f_1 via an electron excitation from electronic ground state S_0 to state S_2 . Transition 2 is the internal conversion between the S_2 and S_1 electronic states. Transition 3 is the vibrational relaxation of the electron from a high vibrational mode of electron state S_1 to a lower one. Transition 4 is a possible radiative fluorescence emission of photon with frequency f_4 via the decay of the electron to the ground state S_0 . Transition 5 is a possible non-radiative decay to the ground state S_0 . Transition 6 is the intersystem crossing to a triplet state T_1 . Transition 7 is a phosphorescent emission of photon with frequency f_7 . Transition 8 is the possible non-radiative decay from the triplet state T_1 to the ground state S_0 .

The black lines in the Jablonski diagram in Figure 3.1 correspond to possible available energy levels for an electron to reside in. The fine lines refer to vibrational excited states for each singlet electronic level S_n and the triple state T_1 . Solid arrows indicate a transition that involves the interaction (absorption/emission) with a visible photon, while dashed arrows indicate non-radiative transitions. In a fluorescent system a path of absorption, relaxation and decay allows for repeated fluorescent emission in a cycle. This begins with ① the absorption of an incident photon of energy $E_1 = hf_1$ by an electron situated in the ground state S_0 . This excites it up to a higher energy level in this case one of the vibrational modes in the electronic state S_2 . The absorption process is extremely fast, happening over a time period of $\sim 10^{-15} s$. This is followed by the internal conversion ② between the levels S_2 and S_1 which is a non-radiative transition where the energy lost by the electron goes into heating the molecule and its surrounding environment. The vibrational relaxation ③ is another possible non-radiative transition, however, between the vibrational states of a single electronic energy level. Both vibrational relaxation and internal conversion transition happen over a time period of $\sim 10^{-14} - 10^{-11} s$. Through decay to the ground state ④ it is possible for the system to release a photon of energy $E_2 = E_{S_1} - E_{S_0} = hf_2$. This is a fluorescent emission for which the transition time period is of the order $\sim 10^{-8} s$, however, this varies depending on the emitting molecule. The energy of the emitted photon is always lower than that of the incident photon because of the energy lost by the electron into the materials via the non-radiative internal losses, which means the emitted light is of a longer wavelength than the incident light. At the same time, it is possible instead for the system to return to the ground state via a non-radiative transition ⑤. This can be through the internal losses of the molecule which relates to its intrinsic quantum yield but also due to some external quenching influence. Once returned to the ground state it is possible for the system to be re-excited and repeatedly emit fluorescent light. This fluorescent emission happens over the course of 10s of nanoseconds, allowing for many tens of thousands of photons to be emitted every second from a single fluorescent molecule.

If a single fluorescent molecule is imaged with the correct integration time, a commonly observed phenomenon is fluorescent blinking. This displays itself as intermittent periods

of inactivity from the molecule where it becomes dark. This is explained by the possibility of a spin conversion transition from the excited singlet states to a triplet state called intersystem crossing (6). The decay period for the triplet state is $\sim 10^{-4} - 10^2 s$, much longer than of the fluorescent decay. While stuck in this triplet state the system is unable to fluoresce. The radiative decay of the triplet state is described as a phosphorescent emission with energy $E_3 = E_{T_1} - E_{S_0} = hf_3$ (7). The difference between the time periods for each emission is due to the decay between the triplet and singlet state being a forbidden transition because of the electron's spin. It is also possible to go from the triplet state back to the ground state via a non-radiative transition (8). This collection of transition times are taken from [34, 35]

After several thousands of cycles of excitation and emission, common fluorescent molecules will become permanently dark through a process called photobleaching. This is often due to a photochemical change of the molecule itself making it permanently unable to fluoresce. The mechanism is not well understood as it is very difficult to study inactive fluorescent molecules but is commonly due to some chemical destabilisation of the molecule such as oxidation.

There are several intrinsic properties of the fluorescence of a single molecule that will be helpful to define. First we define the intrinsic quantum yield (QY), η_0 , which is the ratio of the number of fluorescent photons emitted to the number of photons absorbed for a molecule in free space. As the decay rates for transitions from each state indicate the probability that a particular decay will occur when compared to the other possible transitions, we can use decay rates to define quantum yield [36]:

$$\eta_0 = \frac{\gamma_{r0}}{\gamma_{r0} + \gamma_{nr0}} \quad (3.1)$$

here γ_{r0} is the intrinsic radiative decay rate of light to the far-field and γ_{nr0} is the intrinsic non-radiative decay rate associated with the internal losses of the molecule. We can also define a decay rate:

$$\gamma_0 = \frac{1}{\tau_0} = \gamma_{r0} + \gamma_{nr0} \quad (3.2)$$

where τ_0 is the intrinsic lifetime of the excited state of a fluorescent molecule.

This allows us to rewrite equation (3.1) as:

$$\eta_0 = \frac{\gamma_{r0}}{\gamma_0} \quad (3.3)$$

Because the lifetime of fluorescent molecules can be very short, measurements of it require very specific detection equipment. However because of the extremely useful information on the internal workings of the fluorescent system encoded in it, these measurements have become a very common method of fluorescence characterisation, with standard equipment being commercially available [37].

If we combine the QY and the intrinsic absorption cross section σ_{abs} for a fluorescent molecule we can write the fluorescent excitation cross section σ_{ex} [38]:

$$\sigma_{ex0} = \eta_0 \sigma_{abs} \quad (3.4)$$

here σ_{abs} is the absorption cross section of the molecule in question in $[\text{cm}^{-2}]$. This is a useful metric for comparing the brightness of fluorescent molecules to be used in experiments. The greater the excitation cross section the greater the fluorescence emitted from the molecule for a given illumination intensity. This is illustrated by the equation:

$$F_T = \frac{I_{ex}}{E_{ex}} \sigma_{ex} \quad (3.5)$$

where F_T is the total rate of fluorescent photons emitted $[s^{-1}]$, I_{ex} is the excitation power density in $[W \cdot \text{cm}^{-2}]$ and E_{ex} is the energy of the excitation photon $[J]$ where $E_{ex} = hf_{ex}$ and f_{ex} is the frequency of the excitation photon

As of yet we have only considered the total fluorescence emission from a molecule, however, to take measurements of the fluorescence it is common to collect the light

through a microscope objective. This means that the intrinsic collection rate of fluorescence F_0 is subject to the detection efficiency of the optical setup η_{det} :

$$F_0 = \frac{I_{ex}\eta_{det}}{E_{ex}}\sigma_{ex0} \quad (3.6)$$

Here η_{det} encompasses the collection efficiency of the objective, the transmission efficiency of any optics behind the objective and the quantum efficiency (QE) of the CCD camera used for the measurements.

As we can write that $I_{ex} = cn\epsilon_0|E_0|^2/2$, for which we will collect the constants into $A = cn\epsilon_0/2E_{ex}$, substituting into equation (3.6) and expanding σ_{ex0} we can then write:

$$F_0 = A|E_0|^2\eta_{det}\eta_0\sigma_{abs} \quad (3.7)$$

The increase in fluorescence from a single molecule due to an increase in illumination intensity is simply explained by considering the excitation and emission cycle of the molecule. When imaging a single fluorescent molecule a common integration period used is of the order of 10s of milliseconds [20, 39-44], with the integration time used through this work being 100ms. Because the time taken for a molecule to go from initially being excited to emitting a fluorescent photon is dominated by the decay time of the fluorescent transition, it takes only a few 10s of ns to relax back to the ground state. This means that, assuming a QY of one, a common fluorescent molecule has the ability to emit millions of photons in a single 100ms integration period. However, this number is greatly curtailed by the molecules' resting time between one emission event and the next absorption. This rest period is simply inversely proportional to the probability that a molecule will absorb an incoming illumination photon. By increasing the excitation illumination, I_{ex} , the likelihood of successful absorption is increased, reducing the rest time in the ground state between emission and absorption which, in turn, means that molecule is able to cycle more times in a given integration period, increasing the fluorescent light collected [45]. This can increase the collected fluorescence from a material up to a saturation point, past which a further increase of

illumination power density will give no marked increase in fluorescent emission. The linear and saturation phases of fluorescent molecule emission are demonstrated in Figure 3.2.

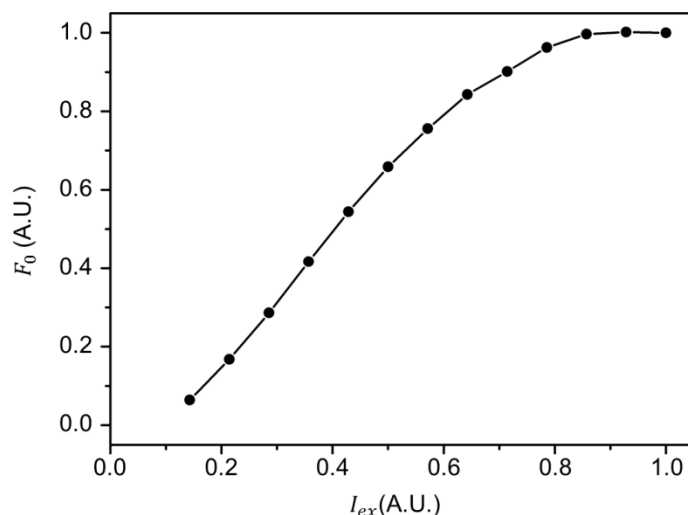


Figure 3.2 – **Laser excited fluorescent emission response.** Normalised collected fluorescence response due to 640nm laser illuminating a film of fluorescent molecule Rhodamine 800.

The linear fluorescent response phase continues from $I_{ex} = 0$ to ~ 0.7 past which it begins to saturate. Figure 3.2 was produced using a film of Rhodamine 800, upon a glass cover slip, illuminated with a 640nm continuous wave (CW) laser. The fluorescence was collected via a 1.49NA 100x oil immersion objective and measured using an EMCCD camera.

The reason for the saturation is simply that the inactive period between fluorescent emission and a new absorption event has been reduced to a minimum. As such, as soon as the molecule is able to absorb, because of the very high power density of illumination, it is immediately supplied with another excitation photon. Thus further increase of power density results no increase in fluorescent emission.

3.2 Single Molecule Fluorescence

Detection of single molecules in any guise has always been technically challenging to achieve. Using molecules which fluoresce allows relatively easy identification of single molecule activity, however, given the low light levels, high sensitivity imaging equipment is needed. Techniques that rely on the observation of single fluorescent molecules have

improved as detection equipment has become more sensitive. Using an electron multiplying charge coupled device (EMCCD) camera in a wide field fluorescence microscope setup, single fluorescent molecule observation has become routine. Figure 3.3 a) illustrates such a setup. It is an example of a total internal reflection (TIR) illumination microscopy setup which is further explored in section 6.1 Optical Setup. This setup gives optical sectioning limiting the illumination to a thin slice extending up to $\sim 200\text{nm}$ above the sample surface. Thus molecules in the liquid outside the illumination plane are not illuminated and they remain dark, reducing the background fluorescence of the measurements. A fluorescent molecule emission is very similar to the emission of a dipole [34]. This means that the amount of light collected from the molecule depends on the orientation of the dipole. The effect is twofold, first, that the excitation of the dipole depends on its alignment with the electric field of the illumination light and second, that the emitted light is only collected if the angle of the light is within the range of the objectives collection capability. The collection angle of the objective is θ . The more aligned with both field and collection the brighter the molecule will appear when imaged. The dipole alignment relationship can be written here as:

$$I_{\text{tot}} = |\mu|^2 |E|^2 \cos^2(\varphi) \quad (3.8)$$

Where I_{tot} is the total fluorescence emitted by a dipole with dipole moment μ and an angle φ to the electric field.

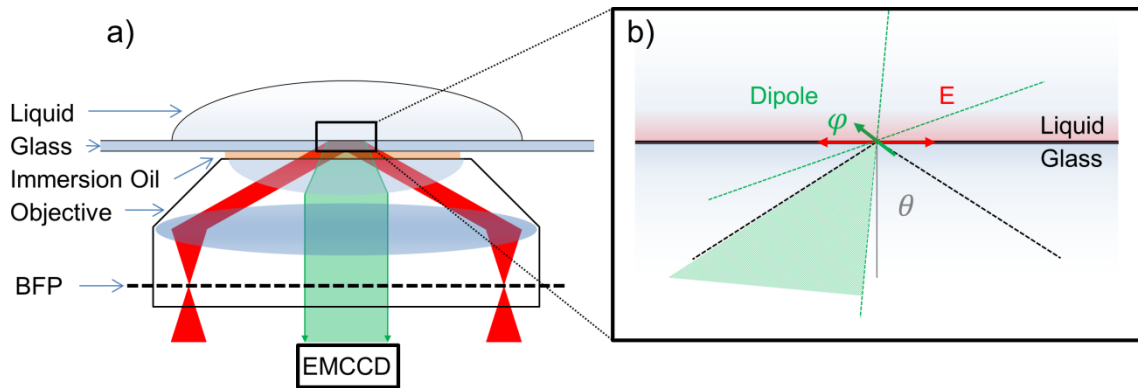


Figure 3.3 – **Schematic for single molecule fluorescence collection.** a) Collection setup using high numerical aperture index matched TIRF objective. Collected light is filtered and imaged by an EMCCD camera. BFP is the back focal plane of the objective. b) Fluorescent dipole emission example for a molecule adsorbed to the Liquid/Glass interface shown in a). φ is the angle between the dipole and the polarization of the illumination field E . θ is the angle of acceptance of the objective in a) light emitted by the dipole at angles greater than this will not be collected.

When we image many thousands of molecules interacting with the surface over time we produce a stack of images as shown in Figure 3.4 a). By choosing the concentration of the fluorescent molecules in the liquid the rate of surface interaction can be adjusted. The number of photons contributed by each interaction were counted and a histogram showing the variation of the fluorescence is shown in Figure 3.4 b).

The properties of single fluorescent molecule make them ideal candidates for various characterisation techniques. This work will mainly focus however on their use as special markers and local field probes.

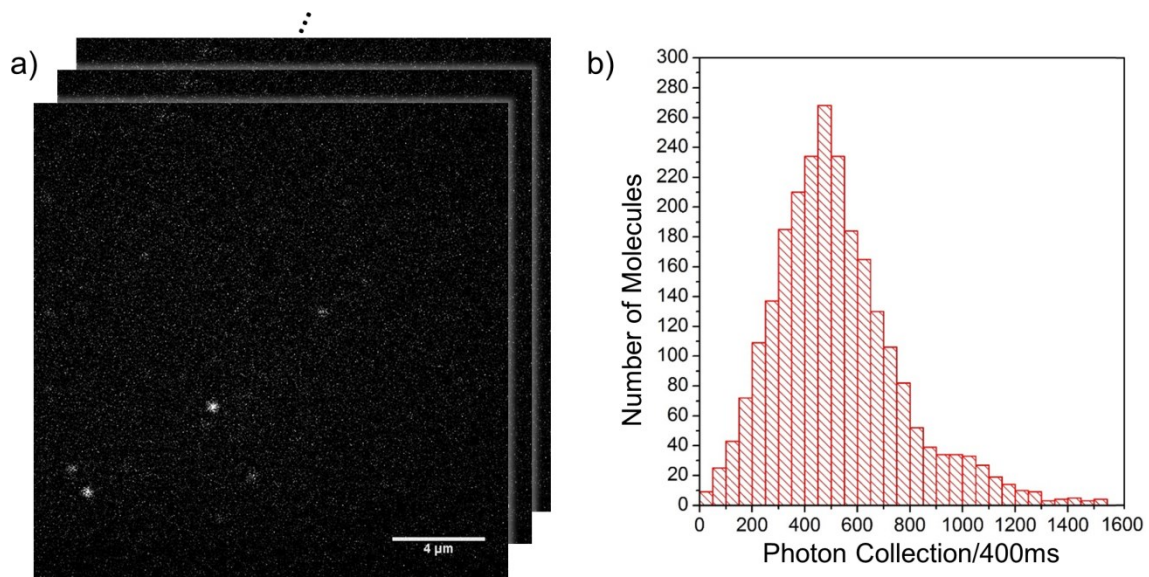


Figure 3.4 – **Demonstration of single molecule interactions at a water/glass interface.** a) Fluorescence image sequence collected from single Rhodamine 800 molecules illuminated with a 640nm CW diode laser. Integration time of 400ms. b) Chart showing the number of events observed with in each fluorescent emission rate range.

3.3 Plasmon Enhanced Fluorescence

When introducing fluorescent material into a plasmonic system an enhancement in its emission when interacting with a suitable plasmonic structure can be observed [6]. We will define the fluorescence enhancement S as the ratio between the collected enhanced fluorescence F_{en} of a material interacting with a plasmonic system and the collected fluorescence of the material without plasmonic interaction.

$$S = \frac{F_{en}}{F_0} \quad (3.9)$$

When we consider the enhancement of a fluorescent emitter, we can split the total enhancement effect into a combination of three things;

- Absorption enhancement
- Emission enhancement
- Quenching effects

As introduced in chapter, 2. Plasmonics, illuminated structures generating LSPR or SPP are able to squeeze the mode volume of light to deep sub-wavelength scales giving rise to large EM near-field power densities. When an emitter is placed within these enhanced fields it can experience illumination intensities many times that it would experience through plain illumination without the plasmonic structure. Assuming that the emitter is not in a saturated state, then the fluorescence collected will increase with the field strength [46, 47] as seen in Figure 3.2. This will be referred to as the absorption enhancement. If the local field around the emitter is increase to saturation point, through illumination increase or increase plasmonic near-field squeezing, there will be no more absorption enhancement for further local field increases.

Spontaneous emission, absorption and stimulated emission can be described using a black body radiation approach taken by Einstein in the early 20th century. The description was very successful for common cases and used rates to describe each action, where the absorption and stimulated emission rate is dependent only on illumination intensity. If Einstein's work was fully descriptive, any enhancement caused by the presence of a NP would be due solely to the focusing effect of the NP on the incident EM field. It would be as if the emitter was just illuminated more strongly. However, in 1946 Edward Purcell et al. published a paper documenting the modification of spontaneous emission through changes in the emitters surrounding environment [48]. This effect of environment on emission is now called the Purcell effect. In this way LSPR act similarly to reflective cavities in which specific wavelengths are limited or selected by the geometry of the

cavity. The emission enhancement of a fluorescent molecule is a feature of a plasmon's effect on the local density of states (LDOS) of space in its vicinity [3, 49, 50]

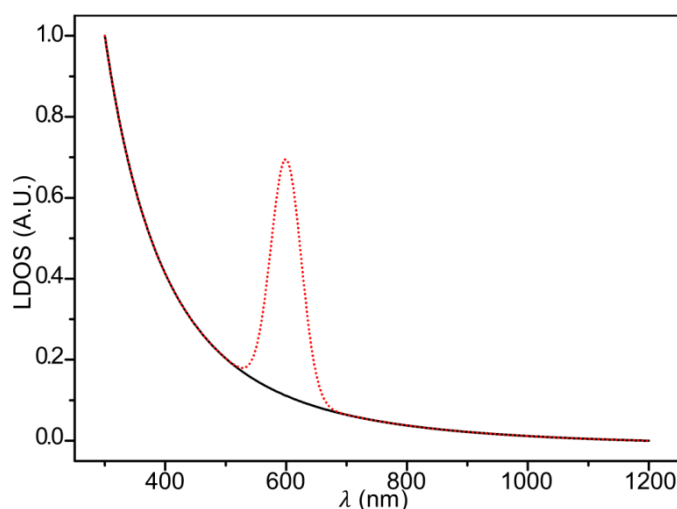


Figure 3.5 – **LDOS Schematic**. A schematic diagram of the difference between the LDOS of free space (black line) and the LDOS interacting in proximity to a plasmonic resonator whose peak resonance is at 600nm.

When an excited emitter relaxes to the ground state via emission in free space, the photon is discharged into one of the states available to it. The number of states available is a function of the wavelength of the photon to be emitted (Figure 3.5 black line). This number of states is known as the density of states with the black line showing the variation for free space. The number of states can be affected by the presence of a suitable plasmonic resonator. An emitter in close proximity will now have access to the plasmon modes as possible states to be utilised for emission (Figure 3.5 red line). The plasmonic modes contribution to the density of states is illustrated by the peak in the red dotted line. The increased number of states makes it easier to emit the photon, reducing the lifetime of the excited state and increasing the probability of a fluorescent emission instead of a dark transition back to the ground state. For this to be most effective the resonance of the plasmon must be close to the wavelength of the fluorescent emission. If this is the case, the fluorescent molecule's QY can be increased as more excitations result in fluorescent emission while at the same time, the molecule's fluorescence lifetime will be reduced as it spends less time in its excited state [49, 50]. Both of these effects lead to an increased number of photons collected for a fluorescent

molecule imaged over a given time period as the fluorescence cycle time is reduced and more cycles are successful in generating fluorescent emission [51]. Plasmonic structures also have non-radiative modes. Through a similar process it is possible to couple the molecules emission into these modes which will enhance the non-radiative emission of the dye. This leads to similar reduced lifetime but also reduced QY. At short distances of interaction fluorescent quenching begins to dominate [50].

Quenching refers generally to any process whereby a material's fluorescence is reduced. Broadly this could be due to many things but in the case of a fluorescent materials' interaction with a metallic surface the most important factors are the non-radiative energy transfer to the structure mentioned previously and at very short ranges a direct energy transfer to the surface. We will consider the non-radiative decay rate enhancement separately leaving quenching for this system to be dominated by direct energy transfer. This happens at very short ranges through the direct transfer of excited charges to the metallic surface which stops all fluorescence. This is illustrated by Figure 3.6 which is replicated with permission from [50]. The plot shows the fluorescent emission rate of a Nile blue fluorescent molecule fixed in a PMMA layer illuminated with a 637nm laser as an Au sphere is brought towards the surface. Initially fluorescent response grows with reduced separation up to a point where $z > 2nm$ and fluorescent rate drops sharply. This was more sharply than predicted due to the presence of energy transfer at super short distances.

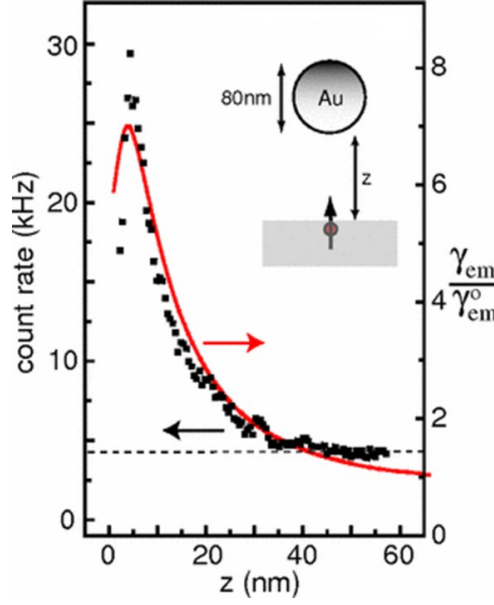


Figure 3.6 - **Fluorescence count rate as a function of distance of Au sphere to surface.** The horizontal grey dashed line indicates the fluorescent background level for molecules whose orientation is shown in the insert. Laser illuminated with $\lambda = 637\text{nm}$ light. Figure was replicated with permission from [50], Physical Review Letters 2006.

If we consider interaction distances between the molecule and the plasmonic system greater than $\sim 3 - 4\text{nm}$ (such that energy transfer quenching effects can be ignored), we are able to rewrite (3.9) in terms of the absorption enhancement f_E and the emission enhancement f_η :

$$S = f_\eta f_E \quad (3.10)$$

The absorption and emission enhancement are expressed as:

$$f_\eta = \frac{\eta}{\eta_0} \quad (3.11)$$

$$f_E = \frac{|\boldsymbol{\mu}_1 \cdot \mathbf{E}|^2}{|\boldsymbol{\mu}_2 \cdot \mathbf{E}_0|^2} = \frac{|\mu_1|^2 |\mathbf{E}|^2 \cos^2(\varphi_1)}{|\mu_2|^2 |\mathbf{E}_0|^2 \cos^2(\varphi_2)} \quad (3.12)$$

where η is the emitter's QE when interacting with the plasmonic structure, η_0 is the free space QE, $\mathbf{E}$ and $\mathbf{E}_0$ are the enhanced and free space local electric field around the emitter respectively, $\boldsymbol{\mu}$ is the dipole moment of the emitter and φ is the angle between emitter dipole moment and the electric field. If we consider only dye molecules whose

dipole moment is aligned with the local electric field we can rewrite equation (3.12) and combine it with equation (3.11) to put equation (3.10) in the form:

$$S = \frac{\eta}{\eta_0} \frac{|E|^2}{|E_0|^2} \quad (3.13)$$

This is the key equation from this chapter which, when combined with simulation data, can be used to calculate the expected fluorescence enhancement values for various plasmonic systems through analysis of its elements. This can then be compared to experimental data for the same values which can be naturally achieved by normalising any enhanced fluorescence response with a base line fluorescence measurement of that specific molecule in free space.

When a fluorescent molecule interacts with a plasmonic antenna, its QY is changed by the antenna effect on the local density of states (LDOS) available to the molecule. This modifies the initial QY η_0 to a new QY η . This is due to the new radiative and non-radiative decay rates γ_r and γ_{nr} from intrinsic rates. Starting from equation (3.1) it is possible to derive the new η from the initial and new decay rates. This new QY can be written as:

$$\eta = \frac{\gamma_r}{\gamma_r + \gamma_{nr} + \gamma_{nr0}} \quad (3.14)$$

Because γ_0 can be written in relation to the intrinsic decay lifetime via equation (3.2) and using equation (3.1), $\eta_0 = \gamma_{r0}\tau_0$. Rearranging to get $\gamma_{nr0} = (\gamma_{r0} - \gamma_{r0}\eta_0)/\eta_0$ and substituting in $\gamma_{r0} = \eta_0/\tau_0$ after some simplification, we arrive at:

$$\gamma_{nr0} = \frac{1}{\tau_0} (1 - \eta_0) \quad (3.15)$$

Substituting this into equation (3.14) and dividing numerator and denominator by γ_0 we can write:

$$\eta = \frac{\gamma_r/\gamma_0}{(1 - \eta_0) + \gamma_r/\gamma_0 + \gamma_{nr}/\gamma_0} \quad (3.16)$$

Where γ_r/γ_0 and γ_{nr}/γ_0 are the radiative and non-radiative decay rate enhancement factors, respectively.

By using classical microscopy techniques the photoluminescence (PL) of a film of fluorescent molecules covering a plasmonic sample can be collected. Each molecule, in its fixed location, acts as a local field probe. Because of the high concentration of fluorescent probes we lose information from any one individual probe and collect a combination of many probes contributing to one total fluorescent image (see Figure 3.7).

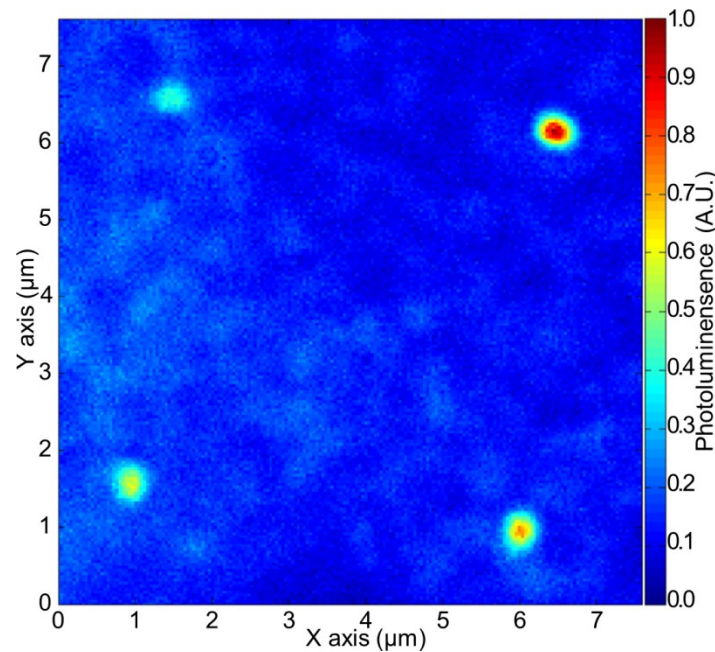


Figure 3.7 - **Wide-field fluorescent microscopy image** of a fluorescent molecule film interacting with plasmonic antenna. Bright spots show enhanced fluorescence due to the interaction of the molecules with the LSPRs of the antenna.

Any structural features of the near-field interaction between the fluorescent molecule and plasmonic structure, shown by the bright patches in Figure 3.7, are lost to the diffraction limited nature of conventional fluorescent microscopy. If we want to map the internal structure of these fluorescent interactions we will need to use a super-resolution approach.

4. Super-Resolution Microscopy

Super-resolution microscopy is the name of a collection of techniques that allow imaging on scales beyond the limits faced by conventional optical microscopy. In this chapter we examine the origin of these limits and the techniques that have been developed to move beyond them, with a focus on localization-based SR techniques.

4.1 Quick History of Diffraction Limited Microscopy

In 1595 Hans and Zacharias Janssen were performing experiments with several lenses that would become the base for the compound microscope. This was improved by Galileo in 1609 and revolutionised the way that humanity looked at and understood the world around them. This initial microscope design would be advanced over the next 250 years or so until Ernst Abbe began his work in optics. Abbe proposed that the maximum resolution of a microscope would be limited by the numerical aperture (NA) of the optics, $NA = n \sin(\theta)$, where θ is the maximum angle over which an optical system can accept light and n refers to the refractive index of the medium that the system is imaging in. This NA controls the minimum size of an optical systems point spread function (PSF) [52].

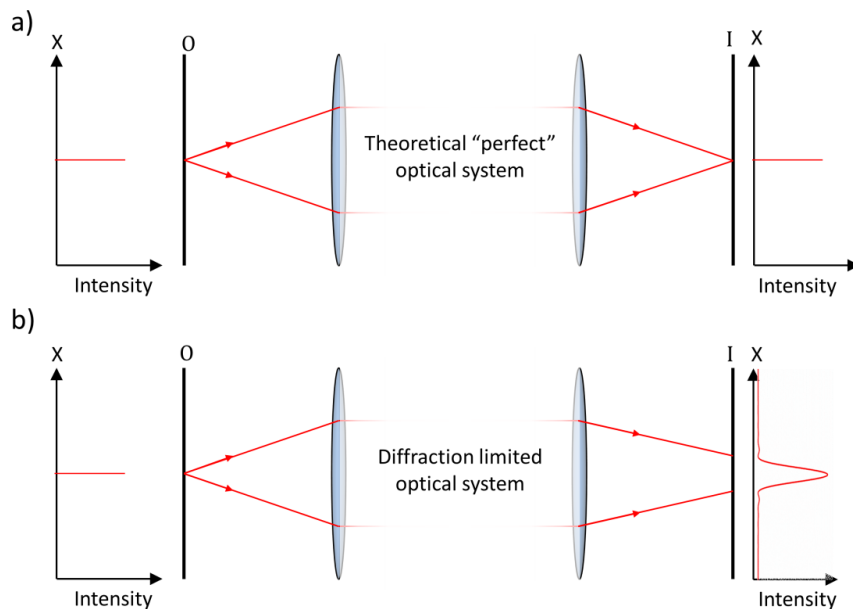


Figure 4.1 – **Schematic of optical systems imaging point sources.** Object planes O containing delta function like point sources. Image planes I containing resultant image projected by optical systems. a) A demonstration of a theoretical perfect optical system. b) A demonstration of diffraction limited optical system.

This PSF of an optical system refers to the minimum spot size projected by an optical system. Generally, this is the size of the image of a point source of light collected by an optical system. For a perfect optical system, we would expect a point source in the object plane to be reconstructed as a point in the image plane which is illustrated in Figure 4.1 a). In a simplistic interpretation, where the image is a product of the convolution of the object and the PSF of the system:

$$Image_{amp} \propto Object_{amp} \otimes PSF \quad (4.1)$$

Where $Object_{amp}$ is the amplitude of the light emitted by the object in the object plane, $Image_{amp}$ is the amplitude of the light of the image created by the system and PSF describes the response of the image system to the point source. If we replace the $Object_{amp}$ with a spatial delta function, it is possible to see how in this limiting case the $Image_{amp}$ becomes directly proportional the PSF as can be in Figure 4.1 b).

Abbe would go on to formulate an equation that proposed an approximate limit to the resolution of an optical system known as the Abbe diffraction limit:

$$d = \frac{\lambda}{2NA} \quad (4.2)$$

where λ is the wavelength of the light being imaged and d corresponds to the resolvable feature size of the system.

The analytical expression of an aberration-free, diffraction limited system with an annular circular aperture that is unobscured is given by:

$$I(R) = I_0 \left[\frac{2\lambda N J_1(\pi R / \lambda N)}{\pi R} \right] \quad (4.3)$$

Where J_1 is a Bessel function of the first kind (order 1), R is the radial distance in the focal plane from the optical axis, N is the f-number of the system and I_0 is the maximum intensity of the produced distribution [53].

When imaging a point source of light with a typical optical microscope, an Airy disk, shown in Figure 4.2, is produced in the image plane. It consists of a strong central peak in which >80% of the light is concentrated surrounded by a series of circular side bands which can be seen in Figure 4.2 a) and d). The central peak is well approximated by the shape of a Gaussian as shown in Figure 4.2 c).

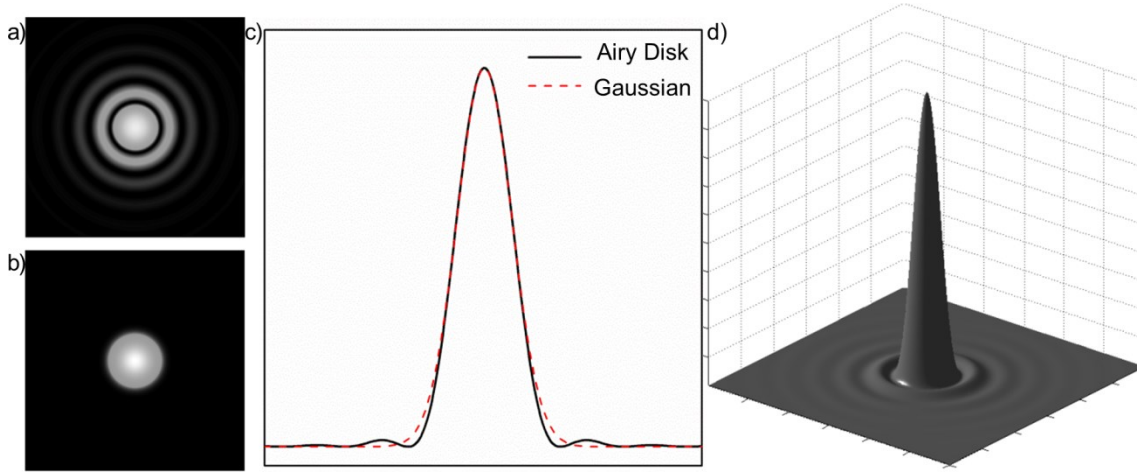


Figure 4.2 – **Example of Airy disk distributions** formed by the diffraction limited imaging of a point source. a) a profile of an Airy disk whose intensity response has been adjusted to clearly display the sidebands. b) profile of a Gaussian distribution whose full width half max is matched to the full width half max of a). c) a central slice through the Airy disk and Gaussian profiles with no intensity scaling d) a surface plot of the Airy disk profile with no intensity scaling.

The resolution of a classical optical system is reflective of the system's ability to resolve features close to each other. Figure 4.3 shows intensity images of point sources of light as they are brought closer together, illustrating how they would look when imaged through an aberration free wide field microscope. Initially in part a) it is easy to see two separate Airy disks corresponding to the two sources. By looking at the corresponding line plot it can be seen that the emitters are positioned over the first side band peak of each other. The emitters then are brought closer together in Figure 4.3 b), where the central peak of each Airy disk is overlapped with the minima of the first trough of the other. The two sources start to become difficult to distinguish. This is the Rayleigh criterion:

$$D_{\text{Ray}} = \frac{0.61\lambda}{\text{NA}} \quad (4.4)$$

Here D_{Ray} is the separation of the two emitters. In Figure 4.3 c) the light sources are positioned closer than the Rayleigh criterion and have become indistinguishable. This is illustrative of the hard limit of a conventional microscope to discern structures with details below the diffraction limit of the light being used to image them. This extends to the conventional imaging of the fluorescence from single molecules which cannot be resolved spatially when arranged close together at distances smaller than Abbe's limit dictates.

From Abbe's proposal of this optical limit, it would be over a century before optical microscopy circumvented the limits proposed by his findings, resulting in a new SR generation of imaging techniques.

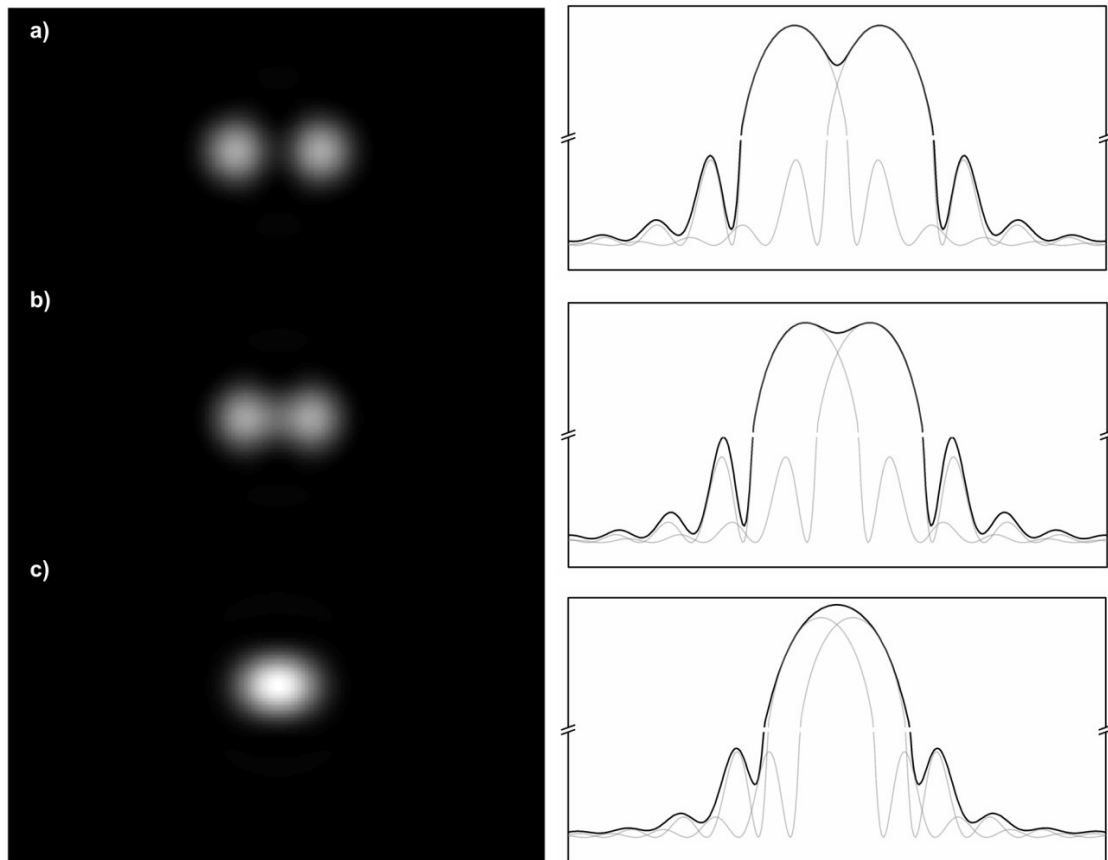


Figure 4.3 –**A demonstration of the diffraction limit of classical microscopy.** The images on the left show examples of point sources of light as they would appear when imaged through a wide field microscope. On the right are shown plots of intensity response in a central slice across each of the left hand images. Plots are made up of two grey lines that show the response of the individual emitters and a black line that shows the combination that is displayed on the left. The y scale of the line plot are separated into two sections at the break line, below which it is plotted linearly and above which, a logarithmic scale is used. This allows the observation of the lateral positions of the side bands. a) shows the distribution for two emitters separated by the distance of the 1st side band. b) shows the distribution for a separation of the 1st trough which is the Rayleigh criterion. c) shows the distribution for two emitters at a sub Rayleigh distance.

4.2 Optical Super-Resolution Techniques

When covering SR microscopy we will break the technical discussing down in to two main groups true SR (TSR) and functional SR (FSR) [54].

TSR techniques are ones that use extra information about an optical system to allow for the direct resolving of structures below the diffraction limit. There are several methods to do this, however, the most prevalent and important are Structured Illumination Microscopy (SIM) and Scanning Near-field Optical Microscopy (SNOM).

SIM is a fluorescence technique that offers a resolution down to approximately 100nm [55, 56]. This is achieved through the introduction of patterning into the illumination light, typically the imaging of a grating [57, 58], instead of the commonly used homogeneous illumination. Frequency mixing of the illumination pattern and the sample through the Moiré effect allows high frequency information to be observed within the frequency supported region of the microscope. This is demonstrated in Figure 4.4 which shows the overlay of a structured illumination pattern Figure 4.4 b) with the image pattern of a small structured Figure 4.4 a). This results in Moiré fringes in Figure 4.4 c)(Figure 4.4 is reproduced with permission from [59] Nano Letters 2010). This high frequency information is then separated from the low frequency information in Fourier space and is replaced into its correct position. By taking several images with different angles of pattern orientation, different parts of the high frequency information that is normally lost can be recovered and replaced. This can then be used to reconstruct the lost high frequency information giving a SIM its increased resolution. SIM however, is unsuitable for our purpose of imaging plasmonic near-field distributions. As plasmonic near-field structures, including hotspots, vary on length scales of 10s of nanometres, they are out of the resolution range of SIM. In addition to this, there is a problem with simply what we want to know about the system. SIM may enable us to image the structure of the plasmonic antenna but we would like to image the structure of the EM-fields generated around the antenna. Furthermore if we were to use SIM on plasmonic structures, we would have to deconvolve, not only the interference of the illumination and reflected light from the structure, but also a complex radiation pattern and

scattering from the plasmonic structure. This would require significant knowledge of the plasmonic system.

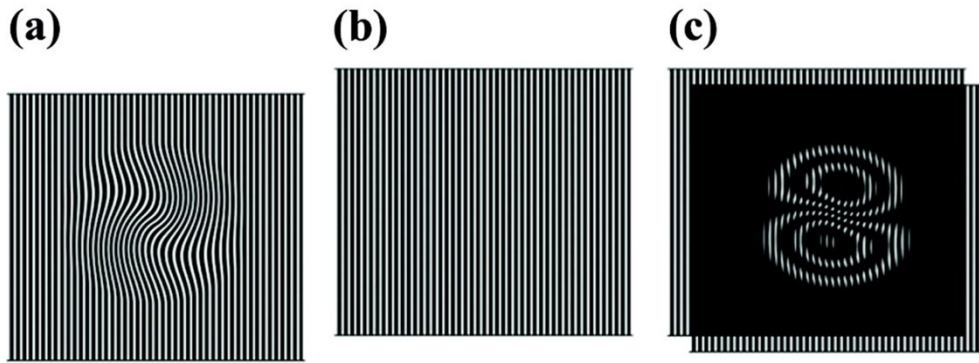


Figure 4.4 - **Demonstration of Moiré fringes used by SIM to access high-spatial frequency information.** a) An example of an object with small features. b) The structured illumination pattern. c) Moiré fringes formed by superimposing structure illumination pattern over the object pattern. This figure was reproduced with permission from [59], Nano Letters 2010.

SNOM uses a more invasive approach. There are several variation on the technique however in a common case SNOM uses a hollow nano tip to illuminate the sample often referred to as aperture SNOM. Light is coupled into the rear of the tip and the nano pinhole at the same end acts to produce a nano scale illumination spot. The resolution of the system no longer scales in the far-field with the wavelength of light but instead the size of the nanoaperture. This tip is often mounted upon an atomic force microscope (AFM) and can then be scanned across the surface of the sample in a point by point manner similar to a confocal microscopy setup. Light is then collected via an objective and although this collected light is diffraction limited in nature it can be attributed to the small area illuminated by the nano tip. Figure 4.5 shows a diagram of how aperture SNOM produces and scans the small illumination area in the near-field of the nano tip across a thin sample. The transmitted light propagates to the far-field to be collected. Figure 4.5 is reproduced with permission from [60]. This gives a PL value at each point which can build up an image of the sample's optical response. More specific details are reserved for the literature [61-65]. On a routine basis resolutions of 50-100nm are achieved with SNOM, however, the technique has been shown to be capable of sub 10nm resolution [66]. Several variations on aperture SNOM have been developed such as scattering methods that use a simple tip to produce the near-field interaction with

the surface [63]. The main limitations for the use of SNOM with respect to the probing of plasmonic near-fields are two-fold. Firstly, commercially available SNOMs are limited to the 50-100nm range with the needed extreme limit of sub 10nm [62] only being really available to heavily customised, bespoke and complex setups. Secondly many plasmonic systems are extremely sensitive to changes in their environment. The presence of the tip in close proximity to a plasmonic system will introduce a non-trivial perturbation of the system's properties. Advantageously for SNOM however, due to this being a non-fluorescence technique, is that no sample preparation is needed and the technique is not hampered by any of the problems of photobleaching.

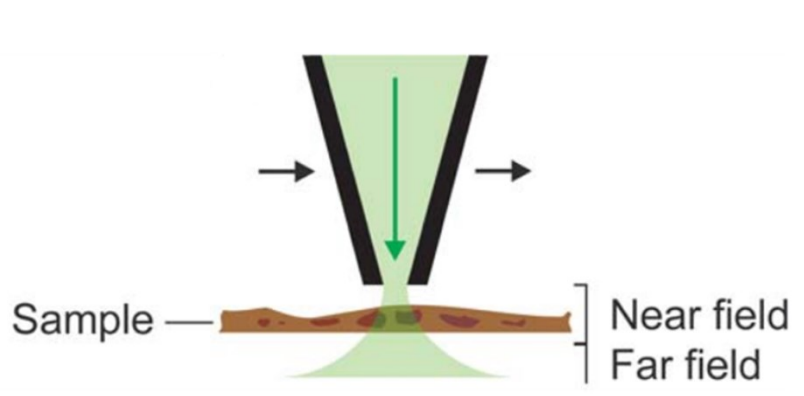


Figure 4.5 – **Example of aperture SNOM.** Light is coupled through a nano tip to illuminate a sample with the focus light in the near-field of the aperture. Light then propagates through the sample to the far-field for collection. This figure was replicated with permission from [60], Nanoscale 2011.

Functional SR techniques do not directly increase the resolution of a microscope. There is no increase in its ability to directly discern objects close to each other. Instead fluorescence is collected from a molecule or material whose active size in object space is known to be sub-wavelength. By assuring sparsity of these active regions any light collected can be assumed to be from the region on the order of the excitation size even through it is spread out when collected in the far-field via the diffraction of light.

Functional SR techniques can be further separated into deterministic and stochastic techniques. The most widely used of the deterministic technique is stimulated emission depletion (STED). Deterministic techniques use a non-linear excitation response for fluorescent molecule or material, which must be affixed to the structures of interest,

such that the excitation volume can be made much smaller than the optical diffraction limit of the system. In the case of STED, this is achieved commonly using two diffraction limited illumination spots, one an excitation spot and one a donut shaped depletion spot. The depletion pulse deactivates the fluorescence of molecules in a ring around the centre of the optical axis such that the active excited region of fluorophores is much smaller than the diffraction limit size. This is illustrated in Figure 4.6 which compares a sample illuminated by a confocal microscope and by a STED microscope. In the confocal case Figure 4.6 a) shows that all the fluorescent molecules within the illumination region are active and fluorescing whereas in the STED case in Figure 4.6 b) only the very central molecules are active as the depletion pulse has suppressed the fluorescence of the surrounding molecule in the illumination region. This gives STED a very small “active” spot in the centre of the illumination beam. This spot is scanned over the sample to build up a fluorescent image of the sample structure. The typical achievable resolution for this technique is 30-80nm [67, 68] however it has been pushed to sub 10nm scales [69]. STED is an optically complex setup to build as the alignment of the excitation and depletion beams is very sensitive and can be challenging to arrange. To work with plasmonic structures adds an extra layer of difficulty as the structures will distort the depletion and illumination beams, affecting the size and shape of the activated fluorescent molecules. This would have to be compensated for in the generation of the beam spots. Lastly STED suffers significantly from photobleaching because of the high powers required. As the fluorescent probes are distributed as a film within the sample they cannot be easily replaced once bleached.

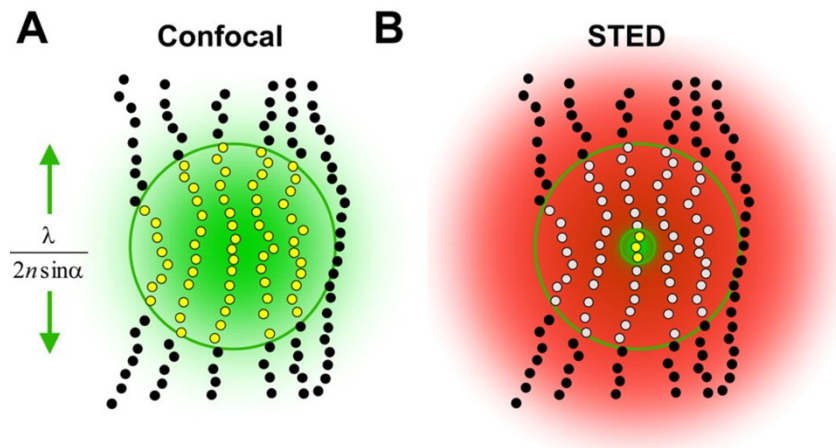


Figure 4.6 – **A comparison of confocal fluorescent response with STED fluorescent response.** a) Confocal microscope illumination of a sample of fluorescent molecules. All molecules within green illumination region are active and fluorescing denoted by the yellow colour. Unilluminated molecules are black. b) STED microscope illumination of the same sample of fluorescent molecules. The green active illumination region is significantly reduced in area with only a small number of active yellow fluorescent molecules. The outer molecules' fluorescence is suppressed, indicated by the grey molecule colour, by the red depletion beam. This figure was reproduced with permission from [70], Circulation Research 2012.

The main stochastic techniques revolve around the localization of single fluorescent molecules imaged in the far-field. This allows the position of a relatively stationary single emitter, that effectively acts like a point source, to be found from the far-field to extremely high precision [71-75]. For this localization to work the emitter must be spatially separated from other emitters in any one image and the large list of different techniques focus mostly on different methods to achieve this spatial separation while still managing to have fluorescent emitters packed densely enough to build an image of the structure of interest. The main techniques are Photoactivated Localization Microscopy (PALM) [20, 76], Fluorescent Photoactivated Localization Microscopy (fPALM) [77], Stochastic Optical Reconstruction Microscopy (STORM)[41] and its variant direct STORM (dSTORM) [39] to name a few. These techniques have been extremely successful in the biological and chemical imaging field [78, 79].

By affixing fluorescent molecules to structures of interest within a biological sample, wide field fluorescent images can be taken of these structures. These images would be made up of light emitted from all the active molecules in the field of view. In conventional fluorescence microscopy these images would be diffraction limited. An

example of this is shown in Figure 4.7 a) which shows a TIRF image of the cell wall of a COS-7 cell. The cell wall appears blurred due to the limitation of the optical system and the diffraction limit of light. Figure 4.7 is replicated with permission from [20]. However, by ensuring the majority of these molecule are inactive, the same image of the system would become a selection of PSFs produced by individual emitting molecules. These PSFs can then be localized giving an accurate coordinate for the position of the molecules. If the active and inactive molecules were then cycled as more images were taken, different sets of PSFs could be localised and over the course of many images, the collection of localizations would build up an image of the tagged structures. This is shown in Figure 4.7 b) c) and d) which are examples of PALM microscopy applied to the same cell which is imaged in part a). The PALM images, made up of individual localized molecules, show significantly increased detail of the cell wall when compared to conventional TIRF microscopy. PALM, fPALM, STORM and dSTORM are all different methods of achieving the inactivity of fluorescent molecules that leads to the sparse images of single molecule PSFs. In the case of PALM photoactivation is used which refers to certain fluorescent molecules abilities to be photoswitched from a common inactive state into an active bright state via a pulse from an activation laser (normally blue shifted from the fluorescent illumination laser). This activates a random selection of molecules which are then imaged until they permanently photobleach rendering them dark again. This is then cycled until enough data is built up to generate a SR image of the structure of interest.

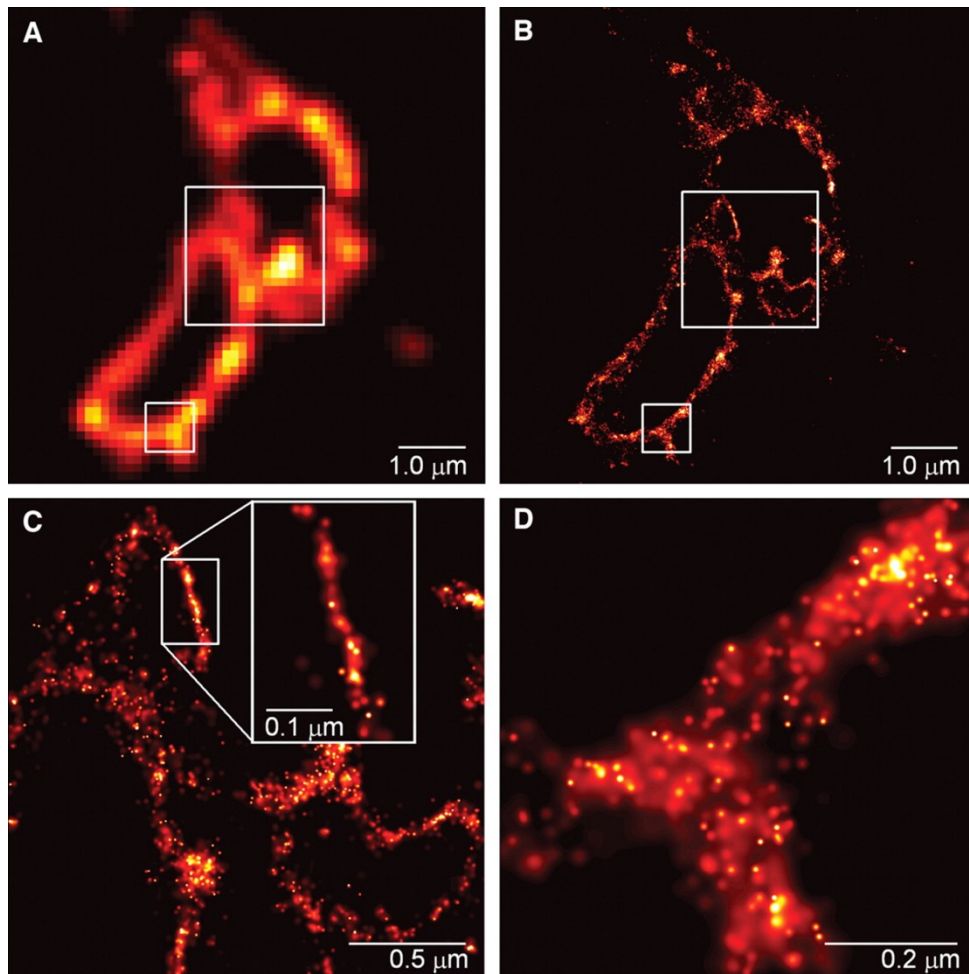


Figure 4.7 - **An example of PALM when compared to TIRF microscopy** of a COS-7 cell whose transmembrane protein CD63 is tagged with the photo activatable fluorescent protein PA-FP Kaede. a) TIRF microscopy of the cell. b) PALM image of the same cell showing fine detail. c) Blow up of large white box from part a) and b). d) Blow up of small box part a) and b). This figure was reproduced with permission of [20], Science 2006.

Finally, we have Point Accumulation for Imaging in Nanoscale Topography (PAINT)[43]. This technique differs significantly from the other listed localization based methods. Instead of using preplaced sources of fluorescence on the structure of interest, PAINT uses transient fluorescent probes that temporarily bind to the sample surface from solution. These stick and produce fluorescence from a fixed location for a short period of time before they are bleached or move off the surface. This fluorescence can be collected and localized. This can then be used over the binding and localization of many probes to map the details of a surface or structure. This was first introduced in a 2006 PNAS paper by Alexey Sharonov and Robin M. Hochstrasser [43]. The paper used diffusing fluorescent molecules to resolve the shape of large unilamellar vesicles as a

demonstration of the techniques effectiveness. PAINT has been applied to other biological systems and was further developed into DNA-PAINT and used to look at the structure of DNA [42]. The technique was not as popular for biological applications as PALM, STORM and their variants, however, it seems ideally suited to be used to probe sub diffraction sized dielectric objects. In 2008 a paper by Dongmin Wu *et al.* worked on this idea [80]. In this work, they imaged PMMA grating structures fabricated on top of a gold film – the gold film being used to act as a contrast material. Only transient molecules that affixed themselves to the PMMA would fluoresce and form localizable PSFs in the image plane, ones that stuck at the gold layer would be quenched through the electron transfer effect. They noticed, however, an unusual effect in some places on the PMMA where they would get extra bright PSF and more frequent localisable interaction. This would lead them to a proposed method of probing plasmonic near-fields discussed in the following section.

4.3 Localization Based Super-Resolution Microscopy

The practise of spatial single fluorescent molecule localization was the product of advancements in the studies of low density lipoprotein receptor molecules (LDL-R) which began around the 1980s. An important paper in the field was a 1982 paper by Barak and Webb [81] in which they used an acetate sheet placed over the video play back on a TV to manually mark and track the movement of these emitters to study the diffusion characteristics along the surface of human fibroblast. This in itself is a type of localization, as by eye the centres of the PSFs are marked, giving a more precise position for the molecule.

With the ability to start digitizing the results of these LDL-R studies papers by Gross & Webb [82, 83], Ghosh & Webb [84, 85] and Gelles *et al.* [86] began to use computational methods for the precise placement of the centroid position. This culminated in a 1994 paper [87] by Ghosh and Webb releasing an automated detection and tracking method to study LDL-Rs by finding the weighted centre of mass of each of the PSFs enabling the tracking of large numbers of particles.

With continued improvements of computational power and the advent of fully digital CMOS cameras the preferred technique for localization imaging became the fitting of Gaussians via a least squares regression (LSR) method [71, 74, 88]. Figure 4.8 shows a 1D example of the fitting of a Gaussian to data collected of the fluorescence from a single Rhodamine 800 molecule.

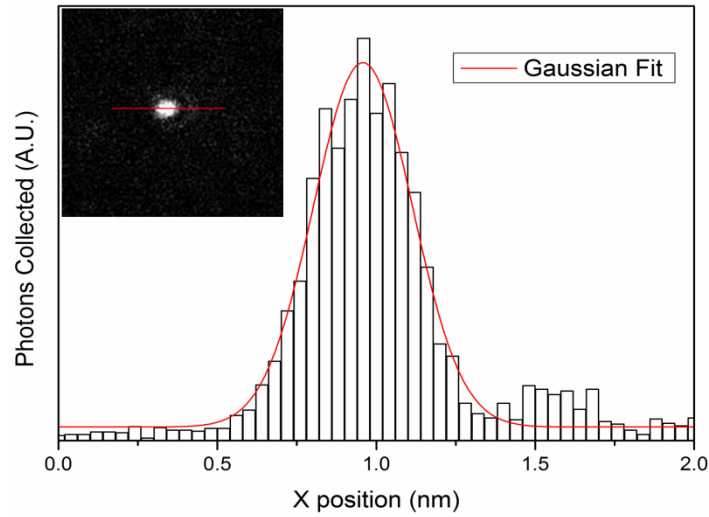


Figure 4.8 - **A 1D example of Gaussian fitting of single molecule fluorescence data.** Each bar contains the total photon number collected in a single pixel along the red line shown in the wide field image insert. A Gaussian was then fitted to the data (shown in red).

The optimisation of the Gaussian fit was improved upon with the introduction of the maximum likelihood estimation method (MLEM) that, for an ideal in focus image of an emitter after pixilation, gives an almost ideal fit when compared to the LSR technique.

For an imaging system of i pixels, n_i counts are made at each pixel. These counts are given an expectation value $E_i(\theta)$ which is a function of the set of parameters θ which describe the Gaussian containing the centroid position. Assuming a Poisson distribution, P_n , for the count numbers n_i , the expectation value for each n written as $\langle n_i \rangle$ will have a set of values θ^* such that:

$$\langle n_i \rangle = E_i(\theta^*) = E_i^* \quad (4.5)$$

This is to say that there should be a particular collection of values θ^* that makes every expected value of n_i have the same values as its E_i counterpart when θ^* is used. It is possible to estimate this set of values by maximising the likelihood function:

$$L(\theta|(n_i)_i) = \prod_i P_{n_i}(\theta) = \prod_i e^{-E_i} \frac{E_i^{n_i}}{n_i!} \quad (4.6)$$

Which has a log likelihood variant:

$$\ell = \ln(L) = \sum_i (-E_i + n_i \ln(E_i) - \ln(n_i!)) \quad (4.7)$$

When the value of ℓ is maximised the set of estimated values $\hat{\theta}$ that give this value described an optimally fitted Gaussian whose centroid point μ is the location of the emitting molecule being localized. These coordinates are contained within the set of values making up $\hat{\theta}$. The variance of the estimated position of the centroidal point is given by:

$$\text{Var}(\mu_x) = \frac{\sigma_a^2}{N} \left(1 + \int_0^1 \frac{\ln(t)}{1 + Na^2 t / 2\pi\sigma_a^2 b^2} dt \right)^{-1} \quad (4.8)$$

Where $\sigma_a^2 = \sigma^2 + a^2/12$ is a correction factor for pixilation effect of pixels with an area a^2 on the width of the Gaussian function, N is the total number of photons, and b^2 is the background photon level at each pixel. A complete explanation of the MLEM and how it compares to other common fitting techniques can be found in Mortensen [71] Nature Methods 2010. The localization software distributed with Mortensen's paper was used throughout this work.

For the MLEM to work, it requires initial estimates for the set of values θ . This includes an estimation of the centre coordinates μ_x and μ_y , an estimation of the width of the PSF σ , the background noise level b^2 and the number of photons contributed by the PSF N . Figure 4.9 gives an example of the processing of a single PSF from raw data to localized point. Raw data is first captured by an EMCCD camera from a single fluorescent Rhodamine 800 molecule on a glass substrate illuminated in TIR with a 640nm CW laser. The raw data is shown as a colour contour plot in Figure 4.9 a) and a surface plot in Figure 4.9 b). This data contains an obvious PSF from the single molecule in the centre. By inputting the necessary estimates of position, intensity, size, and background level we use the MLEM to fit a Gaussian to the PSF. Figure 4.9 c) and d) go on to show the fitted Gaussian first as a colour contour plot then as a wire mesh surface plot. Plotted underneath the wire mesh MLEM fitted Gaussian in Figure 4.9 d) is a surface colour plot

of the centroid position of the fitted Gaussian, this is a second Gaussian whose full width half max (FWHM) is the precision of the initial Gaussians fit. Finally, Figure 4.9 e) displays a colour contour plot of this centriodal Gaussian to demonstrate the dramatic change in spatial size between the original raw data and localised point. For a free space molecule, we can say that the molecule's true location is within this localized point with a confidence of $\sim 95\%$.

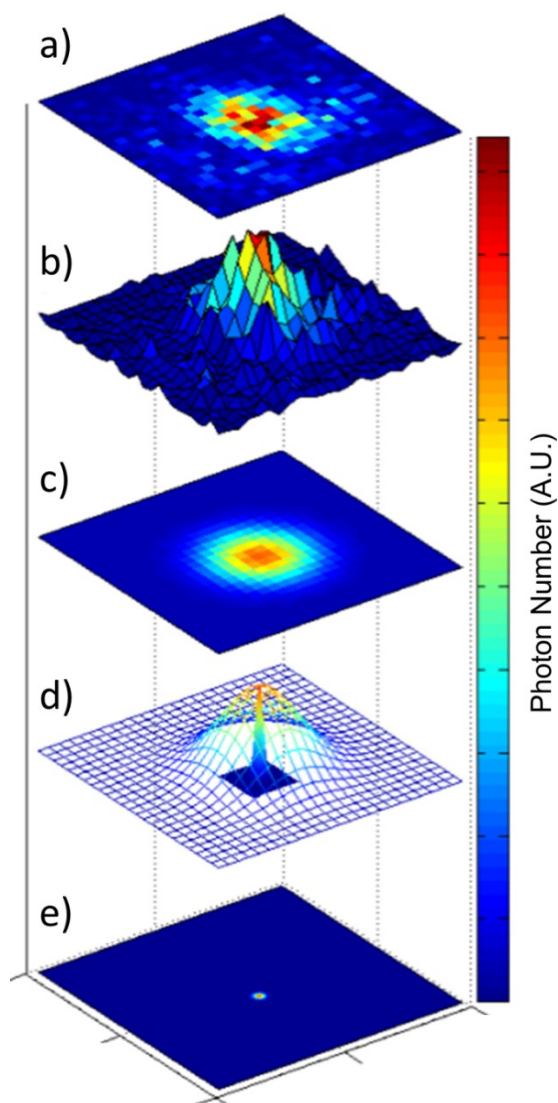


Figure 4.9 - **Example processing of raw fluorescent data from single molecule.** a) An EMCCD camera image is taken. Each pixel contains the number of photons collected. b) This raw data is displayed here as a surface plot which is then fitted with a Gaussian using the MLEM. c) The fitted Gaussian is displayed here as a colour contour plot. d) A surface plot (wire mesh) of the fitted Gaussian is shown here over a solid colour surface plot of a second Gaussian of the centroidal position of the MLEM fit whose FWHM is the precision of the localization calculated via equation (4.8). e) Is a colour contour plot of the centroidal point the solid Gaussian in d).

4.4 Probing Plasmonic Near-Fields Using Localization Based Super-Resolution Microscopy

The intensity of the collected fluorescence from a molecule contains information linked to its local field environment. In this way a single fluorescent emitter can act as a near-field probe. Because of the aforementioned localization technique, it is also possible to find the molecule's position to high precision. This was the realisation made in the Hu Cang *et al.*[89] work from the same group whose PAINT paper was previously discussed [80]. The bright patches that were observed in those results were attributed to the fluorescence enhancement effects of roughness in the gold below a poly(methyl methacrylate) (PMMA) layer. By optimising the conditions, a study of plasmonic hotspot's near-field was produced in 2011.

The PAINT technique is largely unchanged when applied to probe plasmonic structures. A low concentration of fluorescent molecules are dissolved into a liquid, commonly water, and the solution is introduced above the sample of interest. Brownian motion causes the molecules to move about in solution and to collide with the surface of the sample in random locations as shown in Figure 4.10 a). It is then possible for the molecules to be temporarily adsorbed to the surface. These molecules fluoresce in a fixed location and this fluorescence is imaged by an EMCCD camera. These images contain the PSFs from the adsorbed molecules luminescence, (see Figure 4.10 b)) as well as a continuous fluorescent background caused by diffusing molecules which travel too quickly and fluoresce too weakly to be resolved within the exposure time used by the camera. After a period of time the adsorbed molecule will go dark in the images as it will have bleached and/or desorbed from the surface. This leaves this area of the sample free for another molecule to adsorb (Figure 4.10 c)). By allowing this process to happen many times over, a stack of images of surface interactions can be built up. For common PAINT, looking at spatial topography, it is enough to simply localize the positions of all the interactions on the surface and plot these together-giving an image of where on the sample these molecules were able to adsorb. However, to probe the near-field of plasmonic structures, it is necessary to look at the information left in the fluorescence PSF of each interaction. Figure 4.11 shows an example of the data processing for a

plasmonic hotspot produced by a rough 25nm gold film coated in a 4nm layer of SiO₂ using Rhodamine 800 as the fluorescent probe molecule illuminated by a 640nm laser.

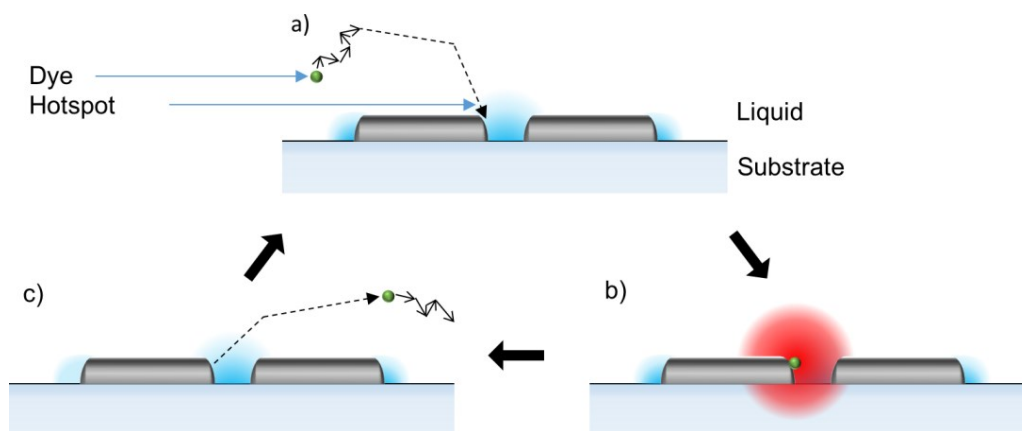


Figure 4.10 – **A schematic of the molecular cycle that allows PAINT style image techniques.** a) Brownian motion of a single fluorescent molecule in a liquid medium allows the stochastic supply of probes to an illuminated sample surface where they are temporarily immobilised. b) The transient molecule fluoresces from its position on the surface. An image of this light is taken with an EMCCD camera which will contain a PSF generated by the diffraction limited collection of the probe molecule's fluorescence. c) The molecule then bleaches and/or desorbs from the surface, leaving the area inactive and free for the next molecule.

As discussed in section 3.2, the collected fluorescence from a single molecule is dependent on its orientation with respect to the collection objective and the electric field orientation. When a molecule binds to the surface its orientation is unknown. Because of this, it is necessary to have multiple probes bind at each location on the sample to allow the true local field conditions to be known. Due to the illumination intensity used, only molecules interacting with strong plasmonic field enhancement are observed within the integration time used for our imaging. The sequence of images, therefore, shows repeated fluorescent interaction at positions over the plasmonic structures see (Figure 4.11 a)). This data is then filtered for inactive frames and the resultant sequence is localized. By plotting this data in a 3D scatter plot (Figure 4.11 b)) it is possible to see how at any one location of the sample there are many individual interactions, each with different amounts of fluorescent light collected. These different values are for the similar dipole orientation response previously mentioned for single fluorescent molecules, however, they are subject to some surface effect on preferred orientations. To recover the fluorescence enhancement influence of the plasmonic

structure itself, the maximum fluorescence for each spatial location must be considered. These corresponded to molecules that were well aligned with the electric field and objective collection angle. Thus the “envelope” of the localization intensity distribution contains the enhancement information. Figure 4.11 c) shows a “skin average” plot of the data shown in part b). The final results are normalised to the unenhanced free space emission from the same dye at a known illumination power to give fluorescence gain values.

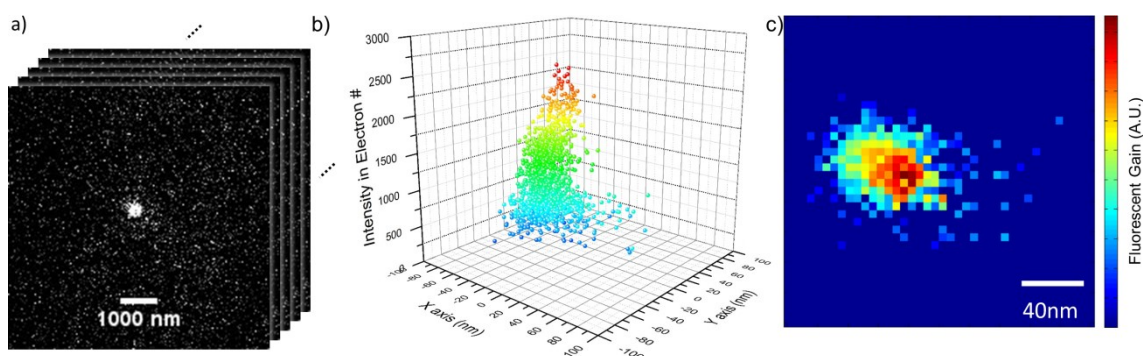


Figure 4.11 - **Data processing of a plasmonic hotspot image stack into fluorescent gain.** a) Fluorescent EMCCD camera image stack for interactions at a hotspot on a gold film. b) Localization data is plotted on an XYZ scatter graph. Each point is a single interaction where xy is the spatial localized position of the molecule and the Z value is the total number of electrons produced in the EMCCD camera for that event. c) Fluorescent gain values for data set.

By probing these near-fields we hope to learn more about how sample geometry and illumination polarization effect the near-field distributions of plasmonic nanoantennas.

4.5 Other Techniques for Probing Plasmonic Near-Fields

Other than PAINT like localization based techniques, there has been recent attempts to use STORM/PALM techniques [90-92] to probe plasmonic near-fields. This is achieved by coating the sample surface in emitters and using fluorescent blinking or forced dark states to realize single emitter fluorescence. The localization of these molecules and study of their fluorescence intensity can map the near-fields of the sample surface. They struggle significantly, however, to achieve high enough emitter densities, while still being able to maintain single emitter fluorescence. This is due to the limitation of the amount of time molecules will spend in dark states. Photobleaching of emitters can also

be a problem here as molecules are fixed in a film so they cannot be replaced once bleached.

Moving away from localization based techniques, work with emitters bound to the end of scanning probe tips has also proved promising in probing plasmonic near-fields [93-96]. This is a scanning based technique where suitable emitters are fixed to the end of an AFM tip allowing the controlled introduction of the emitters as probes into the vicinity of the plasmonic antennas. The antennas are illuminated and the fluorescent light produced by the tip is collected. In this way it is not necessary to localize the position of the fluorescence as the position of the tip is known. Tip fabrication and photobleaching of probe molecules are sources of difficulty for this technique but the high control of the position of the emitter probes may allow for robust studies of the fundamental interactions between fluorescent emitters and plasmonic antennas.

There are a set of non-optical techniques that are also able to provide information about the near-field properties of plasmonic structures which we will cover here.

Electron Energy Loss Spectroscopy (EELS) is an electron beam technique. It uses a scanning transmission electron microscope (STEM) to pass an electron beam through or close by to the plasmonic structure that is the subject. This excites the plasmon via coupling of energy between the beam and the plasmonic modes [19, 97]. The electron beam usually has an energy of between 50-400keV [19]. The energy lost into the plasmonic mode is measured by looking at the energy lost in the transmitted electron beam. By generating an energy loss spectrum for the current position of the electron beam, information of the plasmonic modes at different frequencies becomes available for the current beam position. The beam is then scanned over the sample collecting spectra for each position. This can give detailed images of the plasmonic properties [98-105]. An example of the EELS setup is shown in Figure 4.12 a) which is reproduced with permission from [19]. This electron excited information seems to correspond reasonably with the near-field generated for optically excited plasmonic modes, however, it seems that some features can be absent such as hotspot features [106]. Consequently it appears that more work is necessary to relate the resultant EELS data to EM-field

enhancements, building on the work that has already been done [103, 107]. The high cost of a STEM required for EELS is the main drawback to this method.

Cathode Luminescence (CL) is another scanning electron beam technique. CL in contrast to EELS is SEM compatible [19, 97] as the transmission beam of electrons is not observed. Instead the light emitted by the plasmons generated through electron excitation is collected. The energy of this collected light is recorded but the polarization state can also be recorded. By scanning the electron beam position and recoding the light generated the properties of plasmonic samples can be investigated [108-114]. An example of CL is shown in Figure 4.12 a) and b) for a STEM and SEM setup respectively. As it is not necessary for the beam to pass entirely through the sample, the sample type is not as limited as EELS. However the surface must be coated in a 2nm layer of metal [19]. CL also requires interpretation to gather desired EM field information of the possible optical excitations and work is being done in this area [115, 116].

For a more detailed analysis of both EELS and CL see F. J. García de Abajo 2010 [19] and for how EELS and CL fit in with other plasmonic near-field imaging processes see K. A. Willets 2012 [97].

There are a few other techniques that we will mention here but not cover in detail, such as Photoemission Electron Microscopy (PEEM) [19, 117, 118] which is another electron beam style technique (Figure 4.12 c)) and material growth based techniques[119, 120] where the illumination of the plasmon causes material growth or transition of material over high enhancement areas which can be later imaged with SEM or AFM.

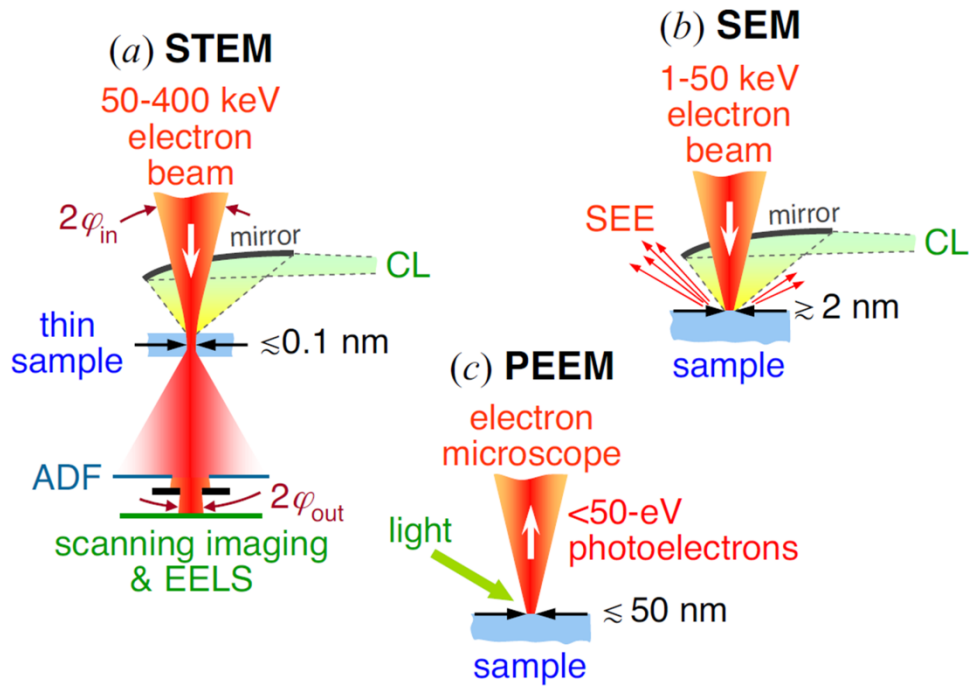


Figure 4.12 – An example of various electron beam spectroscopy techniques. a) A STEM taking EELS and CL measurements of a thin sample ($< 100\text{nm}$). b) An example of a SEM taking CL measurements of a sample. c) An example of a PEEM setup. This figure was reproduced with permission from [19] APS Physics 2010.

5. Finite Difference Time Domain Simulations of Electromagnetic Properties of Plasmonic Antennas

The Finite Difference Time Domain technique is a grid based solver of Maxwell's equations that allows the simulation of the interplay of electric and magnetic fields around an optical subject. By making an approximation for the time derivative component of Maxwell's equations, it is possible to write them in a form that uses finite steps of time Δt , that are extremely short, such that $1/f = t \gg \Delta t$, where f is the frequency the simulated light. These can then be written as a set of updating equations that calculate the new field conditions at time t_2 from time t_1 after time Δt has passed. Maxwell's equations are solved iteratively for every grid point which contains information on the material modelled at that position.

In this body of work we use Lumerical FDTD software to simulate various aspects of plasmonic antenna that are used experimentally. When using Lumerical the subject antenna to be simulated must be drawn to the correct geometry and its material selected from a material data base. This must then be used to fit the response for the material for the refractive index (real and imaginary) for the simulation wavelength range in use. A poor fit of the data can lead to an incorrect/inaccurate simulation, so care must be taken to achieve optimum material properties.

5.1 Plasmonic Scattering Absorption and Extinction Cross Section Simulation

The spectral response for plasmonic antenna is simulated by illuminating the structure of interest with a spectrally broad light source which spans the wavelength range of interest for the structure. The scattering, absorption and extinction cross section are then calculated by monitoring the power transfer through two boxes shown in Figure 5.1. In the diagram the light source is indicated by a black line with the polarization indicated in red and the propagation direction indicated in blue. A special light source type called "total-field scattered-field source" is used for illumination. This splits the simulation space into two volumes. An internal volume (inside the black line), where both illumination fields and scattering fields exist and an external volume (outside the

black line), where only the scattering fields exist. This is to say that the illumination field does not extend outside of the square made up by the black line. This makes simulation of scattering and absorption easier to set up. The object to be simulated is placed on the surface of the substrate and in a medium whose properties are selected before simulation. Lastly the monitors are indicated by the orange lines.

To gather the required information monitor boxes are placed, one internally to the light source and one externally. The external monitor is called the scattering monitor as the power transferred P_{scat} though this will be from light scattered by the antenna. The internal monitor is the absorption monitor as the (negative) power transferred P_{abs} though this will be the absorption of the antenna. These measurements are taken at every wavelength being simulated. To arrive at the cross sections for scattering and absorption the power transfer must be normalised by the source intensity I as:

$$\sigma_{scat} = P_{scat}/I \quad \sigma_{abs} = P_{abs}/I \quad (5.1)$$

Once the scattering and absorption cross section are calculated the extinction cross section σ_{ext} can be calculated using:

$$\sigma_{ext} = \sigma_{scat} + \sigma_{abs} \quad (5.2)$$

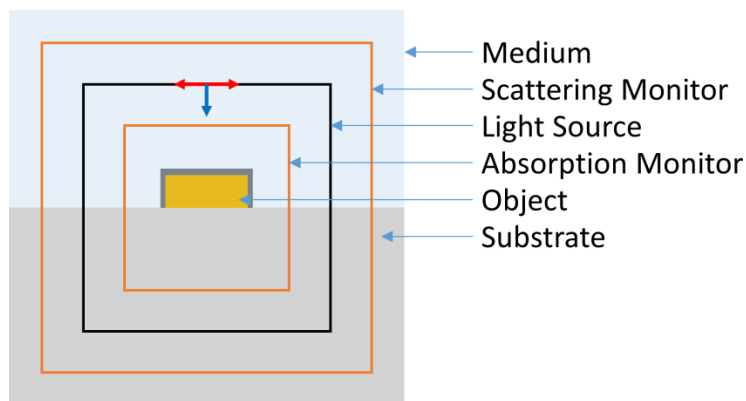


Figure 5.1 – A 2D diagram of the simulation geometry used for antenna spectra response. Where the black lines are the area of the “Total-field Scattered-field” light source with red arrows indicating polarization and blue arrow indicating propagation direction. The orange lines are field monitors. The sample is placed onto a substrate (grey) in a medium (blue).

5.2 Simulation of Electromagnetic Near-Field Distributions

When simulating the near-field distributions for our plasmonic antenna the desired illumination wavelength and polarization is selected for our source in accordance with the illumination wavelength to be used in the experimental setup. The simulation is then run and the resultant electric field is recorded in a stack of horizontal slices in Z. When simulating EM fields around a plasmonic antenna, it is reasonable to only look at the electric field as almost all of the energy of the system will be contained here and not in the magnetic field [1]. The geometry shown in Figure 5.2 a) was used for the early simulation of electric near-fields. The antenna is illuminated with a “total-field scattered-field source” as the data was commonly recorded within simulations made of the scattering, absorption and extinction cross sections.

Later on we explored the TRI illumination geometry used in our experimental setup. To do this the sample was illuminated with an angled plane wave source from below the sample surface. This propagates up from within the substrate, totally internally reflecting at the interface and illuminating the sample with the evanescent fields generated that extended into the medium [121, 122]. This geometry is shown in Figure 5.2 b) where the illumination source is indicated by the black line whose propagation direction is shown by the blue arrow with the polarization shown in red (polarized into the page).

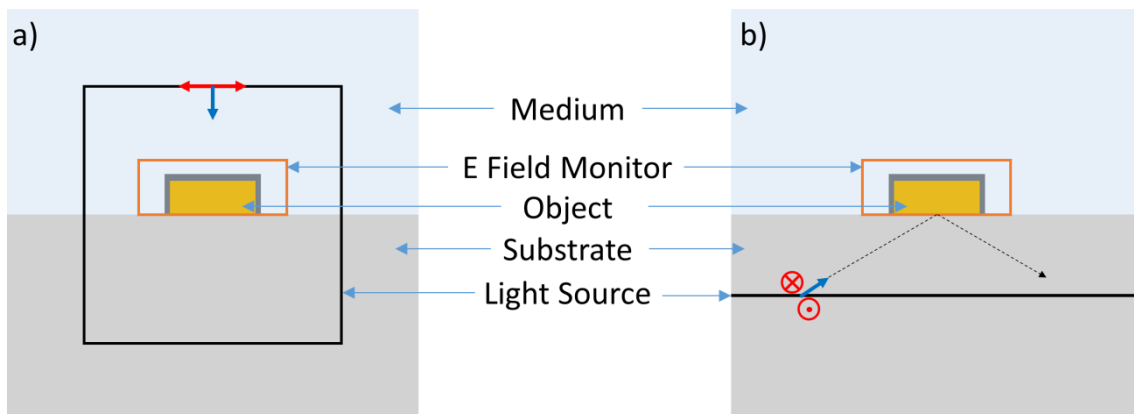


Figure 5.2 - A 2D diagram of the simulation geometry used for antenna EM near-field images. Where the black lines are the area of the light sources with red arrows indicating polarization and blue arrow indicating propagation direction and the orange lines are field monitors. The sample is placed onto a substrate (grey) in a medium (blue). a) Simple simulation geometry using “Total-field Scattered-field” source. b) Sample geometry for TIR illumination near-fields.

5.3 Dipole Interaction with Plasmonic Antenna Simulations

Studying the interaction between emitting dipoles and plasmonic structures is of great interest to investigators using fluorescent molecules as probes. Here we break the study into two parts. First the projection of near-field interactions into the far-field to produce a simulated PSF which allows us to explore the effects of emission interactions with the plasmonic system on the far-field images generated. Second the exploration of emission enhancement on the dipole due to the proximity to the plasmonic system that provides more optical states, radiative and non-radiative, for the dipole to emit into.

To generate a far-field projection of near-field interactions we made use of Lumerical's `farfieldux` and `farfielduy` functions. These project a field profile into the far-field by decomposing the near-field data into a set of plane waves propagating at different angles and calculating the result in the far-field from this.

Figure 5.3 shows the position of the field monitor below the sample surface. This monitor spans the entire simulation space in a plane. This is important as any field lost will reduce the accuracy of the projection. The dipole source position and polarization is indicated by the red arrow. The simulation is run and the electric field is recorded, which is then projected as plane waves using the “`farfieldux`” and “`farfielduy`” functions. This is subsequently filtered through an aperture with a specific NA where $NA=1$ to account for the objective used in experimental light collection. Finally a calculation of the resultant image is made using a chirped z transform. This image can then be localized which gives an “apparent” localization position of the dipole from the far-field data. It is then possible to compare this position with the true position of the dipole. By scanning the position of the dipole in proximity to the plasmonic antenna we can study how the interaction effects localization. An example of the Lumerical script used for far-field projection is given in Appendix B.

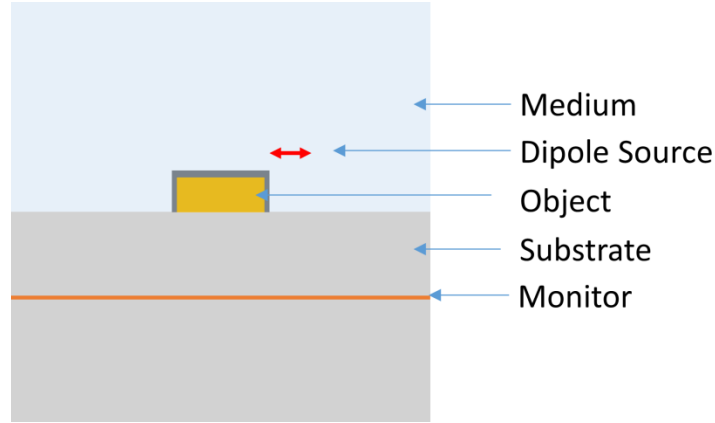


Figure 5.3 - **A 2D diagram of the simulations projecting near-field dipole antenna interactions into the far-field.** Where the red arrows indicate the position and polarization of the dipole light source and the orange line is the field monitor. The sample is placed onto a substrate (grey) in a medium (blue).

The extra available optical states provided by plasmonic nanoantennas can influence the emission of a nearby dipole. To explore this we approach the simulation in two separate ways. One uses direct measurements of power transfer normalised by the dipole's unenhanced emission. The second way is via the calculation of the LDOS for the dipole and from that a calculation of the emission enhancement can be made.

To calculate decay rate enhancement values for radiative and non-radiative modes by direct power transfer monitoring we use the relationship between power enhancement and decay rate enhancement:

$$\frac{\gamma}{\gamma_0} = \frac{P}{P_0} \quad (5.3)$$

Where γ and γ_0 are the decay rates with and without plasmonic interaction and P and P_0 are the power output for the dipole with and without plasmonic interaction. Figure 5.4 a) shows the configuration used to monitor the power transfers needed to calculate enhancement rates. This requires two monitor boxes. The first is around the dipole called the dipole monitor and the second is around the structure called the structure monitor. The power that is transmitted through the structure monitor when normalised for an unenhanced dipole power gives the radiative decay rate enhancement. By subtracting this from the normalised dipole transmission we arrive at the non-radiative decay rate enhancement value.

To calculate the total decay rate enhancement through the LDOS value we use the Dyadic Green's function [2], which, is a matrix where each column refers to the field of an x, y or z orientated dipole. For example let $\mathbf{E}(\mathbf{r})$ be the field at a position $\mathbf{r}$ for an x -polarized dipole source at position $\mathbf{r}_0$ with dipole moment μ , can be calculated as:

$$\bar{\bar{G}}_x(\mathbf{r}, \mathbf{r}_0) = \frac{\mathbf{E}(\mathbf{r})c^2\varepsilon_0\varepsilon_r}{\omega^2\mu} \quad (5.4)$$

Where ω is the angular frequency, ε_0 and ε_r are the permittivity of free-space and the dielectric constant at the point $\mathbf{r}$, respectively. With similar cases for y and z orientated dipoles. In the case of these simulation setups $\varepsilon_r = n^2$.

This expression is simplified and calculated for a specific dipole orientation for each simulation. From this we can calculate the density of states, ρ , for that dipole orientation contribution:

$$\rho_x = \frac{2\omega}{\pi c^2} I[\bar{\bar{G}}_x] \quad (5.5)$$

Here $\bar{\bar{G}}_x$ is the x -polarization component of the Green's function. From this density we are able to calculate the radiative decay rate γ for an x -polarized source:

$$\gamma_x = \frac{\pi\omega}{\hbar\varepsilon_0\varepsilon_r} \rho_x \quad (5.6)$$

Which we normalise through the calculation of the free-space radiative decay rate γ_0 :

$$\gamma_0 = \frac{\omega^3\varepsilon_r^2}{3\pi\varepsilon_0\hbar c^3} \quad (5.7)$$

Figure 5.4 b) shows the simulation setup used for the total decay rate enhancement simulation via LDOS calculation. Note that no monitors are used. Instead various Lumerical script functions, that return the electric field generated by the dipole for the current position and polarization, are used. To build up images of how the enhancements change over the sample the dipole's position is scanned and at each

point x and y polarized dipoles are simulated separately. The results are then built up into an image for a specific emission wavelength. An example of the Lumerical script used for emission enhancement scan simulations is given in Appendix B.

The two techniques calculate the total emission enhancement on one hand and the separate radiative and non-radiative emission enhancements on the other, and can be easily compared to verify each other as:

$$\frac{\gamma}{\gamma_0} = \frac{\gamma_r + \gamma_{nr}}{\gamma_0} \quad (5.8)$$

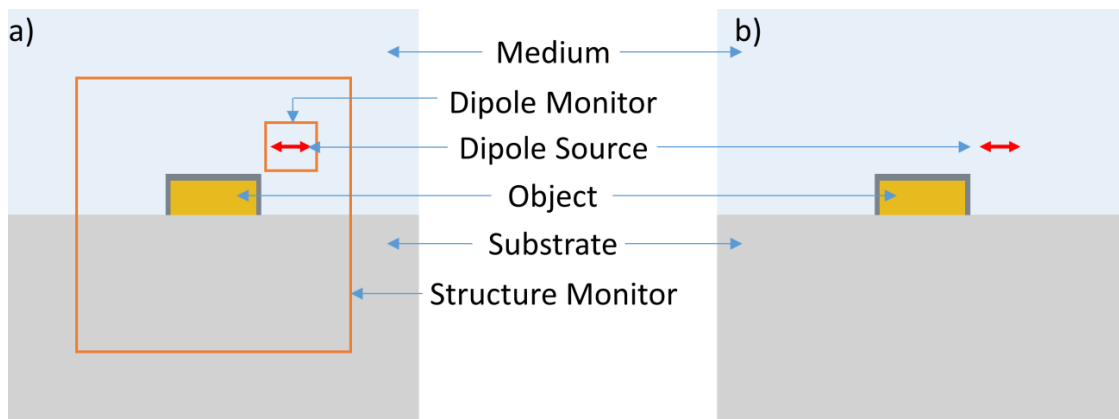


Figure 5.4 - **A 2D diagram of simulations near-field dipole antenna interactions effect on emission enhancement.** Where the red arrows indicate the position and polarization of the dipole light source and the orange line is the field monitor. The sample is placed onto a substrate grey in a medium blue. a) Geometry used for direct power transferred between dipole and structure and the far-field. b) Sample geometry used for emission enhancement via Green's function calculation.

6. Experimental Setup

When designing an experimental setup for single molecule localization based SR microscopy, great care must be taken to ensure suitably high mechanical stability for measurements. As measurements are made over large acquisition cycles, mechanical and thermal drift in sample position will be translated into the cumulative data, unless steps are taken to minimise, or to track and remove any movement from the final data.

Special consideration should also be taken to maximise fluorescent light collected and to minimize the collection of extraneous light that forms the background fluorescence of an image, as the accuracy of localizations are limited by the signal to background noise level in each image (this can be seen in the variance equation (4.8)). This requires the correct selection of objective lens, laser filters, dichroic mirrors and emission filters to minimize unwanted background and maximize the signal collected. This also extends to the fabrication of plasmonic samples. Any materials used should be checked in order to ensure no unwanted light will be generated at the collection wavelengths via the laser illumination.

A basic localization based microscope can be quite simple and cheap to set up, with the major costs being a suitable microscope, scientific camera (most commonly EMCCD over sCMOS) and a high numerical aperture objective. The setup used in this work, however, extends upon this basic setup to include a sample tracking and stabilization system that allows prolonged imaging sequence to be taken, without suffering adverse effect on the resultant localization maps due to sample drift or defocus.

6.1 Optical Setup

The optical setup used was built around a Nikon Ti-U inverted microscope [123]. This microscope was fitted with a Nikon TI-TIRF Illuminator Unit [124] for excitation that was used in combination with a CFI Apo TIRF 100x oil objective (1.49 NA). CW diode lasers of various wavelengths were coupled to the TI-TIRF Illuminator unit via a Qioptiq KinFLEX-HPV polarization maintaining fibre. The sample being imaged was mounted on a piezoelectric stage that itself was fastend onto the mechanical stage of the microscope.

A wide field fluorescent microscope forms the core of the setup using a CW laser illuminated high numerical aperture oil objective to excite and image fluorescence from the sample. A set of filters chosen specifically to work with illumination/emission wavelengths were used to filter out the laser light from the collected light. A schematic of the setup is shown in Figure 6.1. Light is coupled out of the fibre F and the adjustable lens L1 and fixed lens L2 are used to focus the beam to the back focal plane of the objective O through the laser clean up filter LCF. The beam is shifted off axis using a micrometre that can adjust the position of the fibre mount laterally. The result of this is a collimated illumination beam that strikes the surface of the sample at an angle controlled by the offset of the fibre. By increasing the angle of incidence to past the critical angle calculated for the glass/liquid interface the illumination beam will totally internally reflect at the interface. This is called total internal reflection (TIR) illumination.

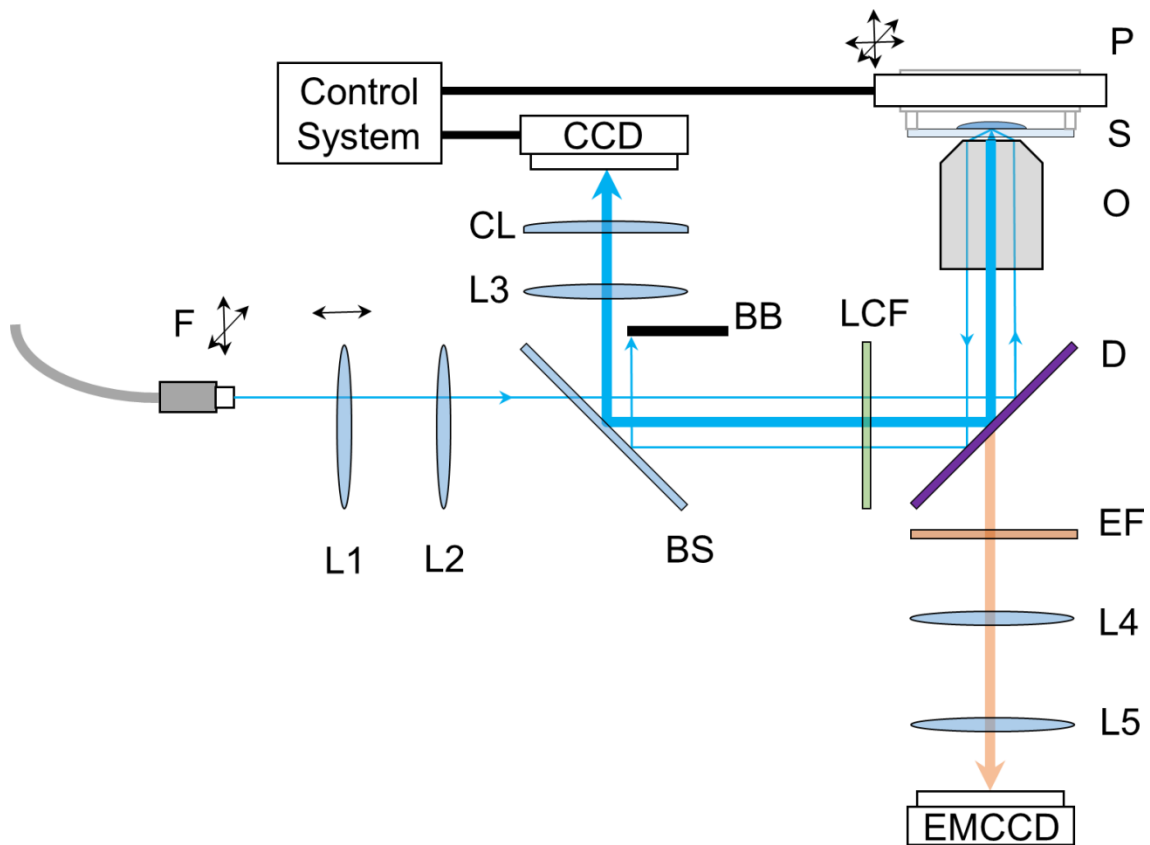


Figure 6.1 – **Schematic diagram of the optical setup used for localization based near-field probing.** F – Optical fibre, L1 L2 L3 L4 L5 – lenses, BS - beam splitter, BB - beam block, CL - cylindrical lens, LCF - laser clean up filter, D - dichroic filter, O - objective, P - piezoelectric stage, EF – Emission Filters.

The benefit of TIR illumination is reduced background fluorescence from material out of focus and away from the surface interface between the substrate and the liquid medium. The evanescent fields produced only penetrate a maximum of $\sim 300\text{nm}$ [121]. The more common epifluorescence illumination technique, shown in Figure 6.2 a), which involves the direct illumination of the sample, illuminates all fluorescent material above the sample surface. This can introduce a large background fluorescence. If the angle of incidence of the illumination θ_I is increased to greater than the critical angle of the interface, which can be calculated as shown in equation (6.1), then total internal reflection will occur limiting the molecules high above the interface from fluorescing.

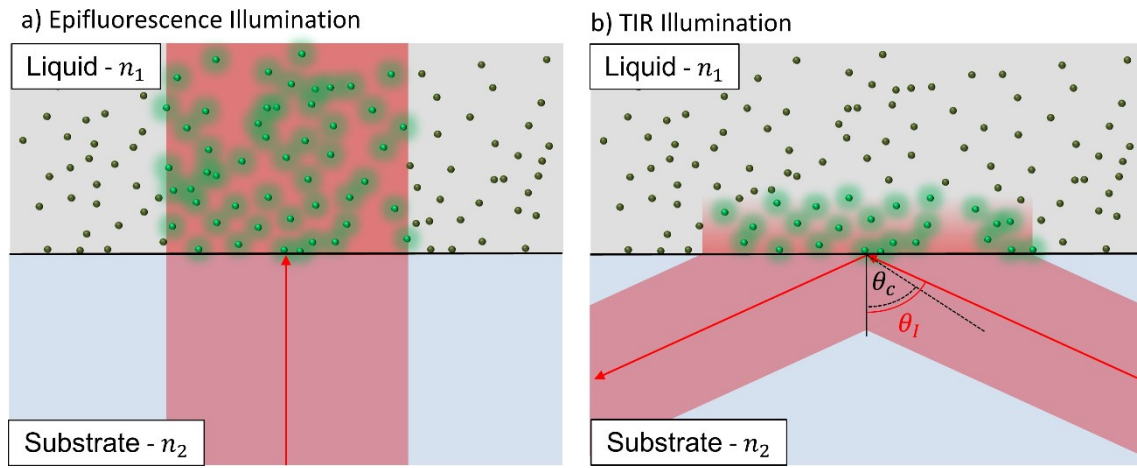


Figure 6.2 - **Schematic of common fluorescence illumination techniques.** a) direct epifluorescence illumination geometry. b) Total internal reflection illumination using incident angle θ_I to achieve optical sectioning.

The critical angle changes with respect to the substrate and medium's refractive index, n_2 and n_1 , respectively, TIR requires $n_2 > n_1$. Higher refractive index liquid mediums mean a steeper angle is needed before TIR illumination is possible for the same substrate. This can make TIR difficult in some cases, and for close refractive indexes between substrate and medium it can be unachievable. The critical angle is given by:

$$\theta_c = \arcsin\left(\frac{n_1}{n_2}\right) \quad (6.1)$$

The intensity of the evanescent field decays exponentially with perpendicular distance from the interface and can be written as:

$$I = I_0 e^{-z/d} \quad (6.2)$$

Where z is the perpendicular distance from the interface, I_0 is the field intensity at $z = 0$, and d is the penetration depth, which is incident angle dependent and can be expressed as:

$$d = \frac{\lambda}{4\pi(n_2^2 \sin^2(\theta_i) - n_1^2)^{1/2}} \quad (6.3)$$

Here λ is the wavelength of the incident illumination. It can be seen from equation (6.3) that as $n_2^2 \sin^2 \theta_i \rightarrow n_1^2$ the denominator of the equation approaches 0 making d extremely large. This limiting case is equivalent to $\theta_i \rightarrow \theta_c$. This is shown in Figure 6.3 a) for two different incident wavelengths, for the case of a glass/water interface with $n_1 = 1.33$ and $n_2 = 1.51$. Using equation (6.1), it is possible to use these values to calculate the critical angle for the system, arriving at $\theta_c = 61.7^\circ$ which is marked on Figure 6.3 on both plots. As expected the shorter wavelength shows a lower penetration depth, both wavelengths have penetration depths of less than 150nm for incident angles larger than 65° .

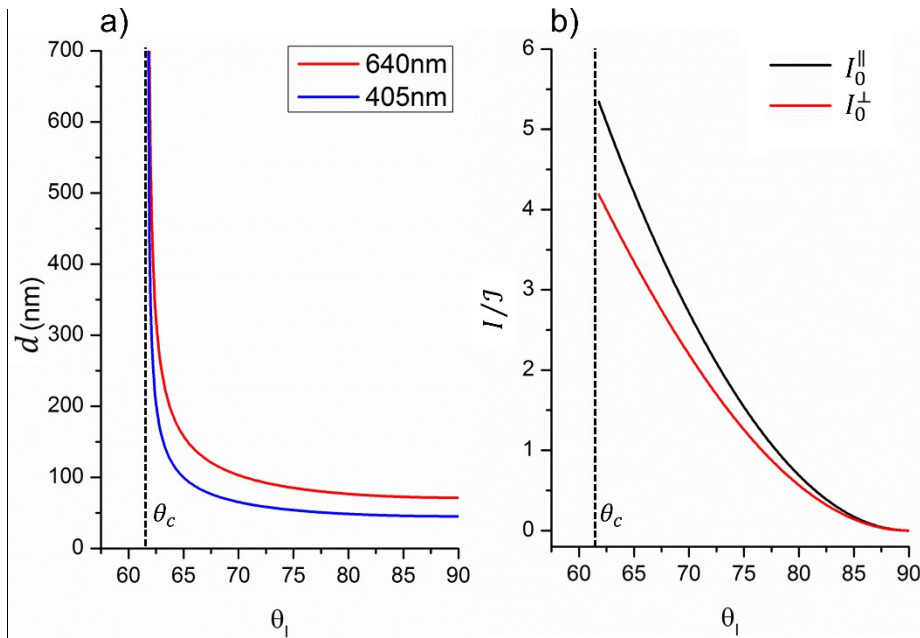


Figure 6.3 – **Plots of TIR generated evanescent field properties for a glass-water interface.** a) Incident angle dependence of penetration depth d for two incident wavelengths 640nm and 405nm. b) Field Intensity normalised by incident field intensity for parallel and perpendicular polarization orientation.

The magnitude of I_0 is not the same as the incident field intensity $\mathcal{I}$ but is related to it via the incident angle and incident polarization. Considering the cases of incident light with polarizations parallel and perpendicular to the interface plane, denoted as $\mathcal{I}^{\parallel}$ and $\mathcal{I}^{\perp}$ respectively, the equations describing I_0 can be written as:

$$I_0^{\parallel} = \mathcal{I}^{\parallel} \frac{4\cos^2(\theta_1)(2\sin^2(\theta_1)-n^2)}{n^4\cos^2(\theta_1)+\sin^2(\theta_1)-n^2} \quad (6.4)$$

$$I_0^{\perp} = \mathcal{I}^{\perp} \frac{4\cos^2(\theta_1)}{1-n^2} \quad (6.5)$$

Where $n = n_1/n_2$. Figure 6.3 b) is a plot of the relative intensity, normalised to the incident intensity, for both polarizations at $z = 0$. For angles close to the critical angle, I_0 is several times larger than the incident intensity. This can be another benefit of using TIR illumination. As the incident angle is increased towards 90° , however, the intensity drops to 0.

Once the sample is illuminated, fluorescence is generated from emitters within the evanescent TIR field. This fluorescence is collected in the EMCCD camera via the dichroic D and emission filters (EF) shown in Figure 6.1. These are selected for the specific fluorescent dye and laser being used, functioning to cut off the illumination light and any background light. A 4x beam expander is placed between the filters and the camera to enlarge the projected image onto the camera's CCD array. The EMCCD camera used to monitor fluorescence is an Evolve 512 (Photometrics) [125]. The camera has a 512×512 array of $16\mu\text{m} \times 16\mu\text{m}$ pixels. With the magnification of the system being around 400x in total, the pixels in image space should each correspond to an area $40\text{nm} \times 40\text{nm}$ in object space. An EMCCD camera works by converting incoming photons into electrons, which are then accelerated, cascading through multiplication section of the camera, generating more electrons until they reach the end and are read out as a signal. The initial conversion of photon to electron has some conversion efficiency that for the Evolve 512 is around 90% for the collected fluorescence in this work [126].

Scattered laser light is collected by a CCD camera (QICAM 12-bit Color Fast 1394) via the monitoring arm of the setup which is split off from the illumination beam path using a 50/50 beam splitter (BS). By tracking the position of the antenna on the sample surface, sample drift can be corrected for by adjusting the position of the sample using the piezoelectric stage. This part of the setup is covered in greater detail in section 6.5.

6.2 Sample Setup and Fabrication

Fabrication of nanoscale antennas is a challenging task. Many plasmonic studies often rely on chemically synthesised metallic structures which allow easy access to plasmonic properties. However, these structures are difficult to manipulate into specific locations and orientations making them unsuitable for highly reproducible surface structure design.

Electron beam lithography allows the fabrication of nm scale metallic structures in a controlled and highly reproducible way. This has made it one of the most important fabrication techniques for plasmonic engineering. Electron beam lithography works very similarly to any other lithographic technique, such as photolithography, in that it uses a resist which is exposed to a pattern. This resist is developed to leave an imprint of the pattern in the resist. This is then used as a mask for the material that is to make up the structures. Electron beam lithography is able to achieve narrower line widths and feature sizes than photolithography as photo-lithography is a diffraction limited technique, making it unsuitable for the length scales we require. EBL suffers from a low throughput, as each line must be individually drawn. In photolithography a full working area mask is used such that all features are exposed simultaneously.

Electron beam lithography resists are materials that, when exposed to an electron beam, chemically change in such that they become resistant (negative resist) to or susceptible (positive resist) to a specific solvent.

For this work a positive electron beam resist - poly (methyl methacrylate) (PMMA) - was used. All samples were fabricated onto 160 μ m thick No. 1 cover slips (VWR). These thin slips are used to match the short working distance of the oil TIR objective used. Figure 6.4 is a schematic of the electron beam lithography process used to manufacture

samples for this work. Before fabrication, the glass slides were cleaned by submersion and sonication in acetone followed by an isopropyl alcohol (IPA) and deionised (DI) water rinse. This reduces the presence of any contaminants on the surface of the glass (Figure 6.4 a), which improves fabrication consistency and material adhesion. Slides are dried post-cleaning by flowing nitrogen over the surface from a compressed nitrogen gun. This is the standard cleaning procedure used throughout this work.

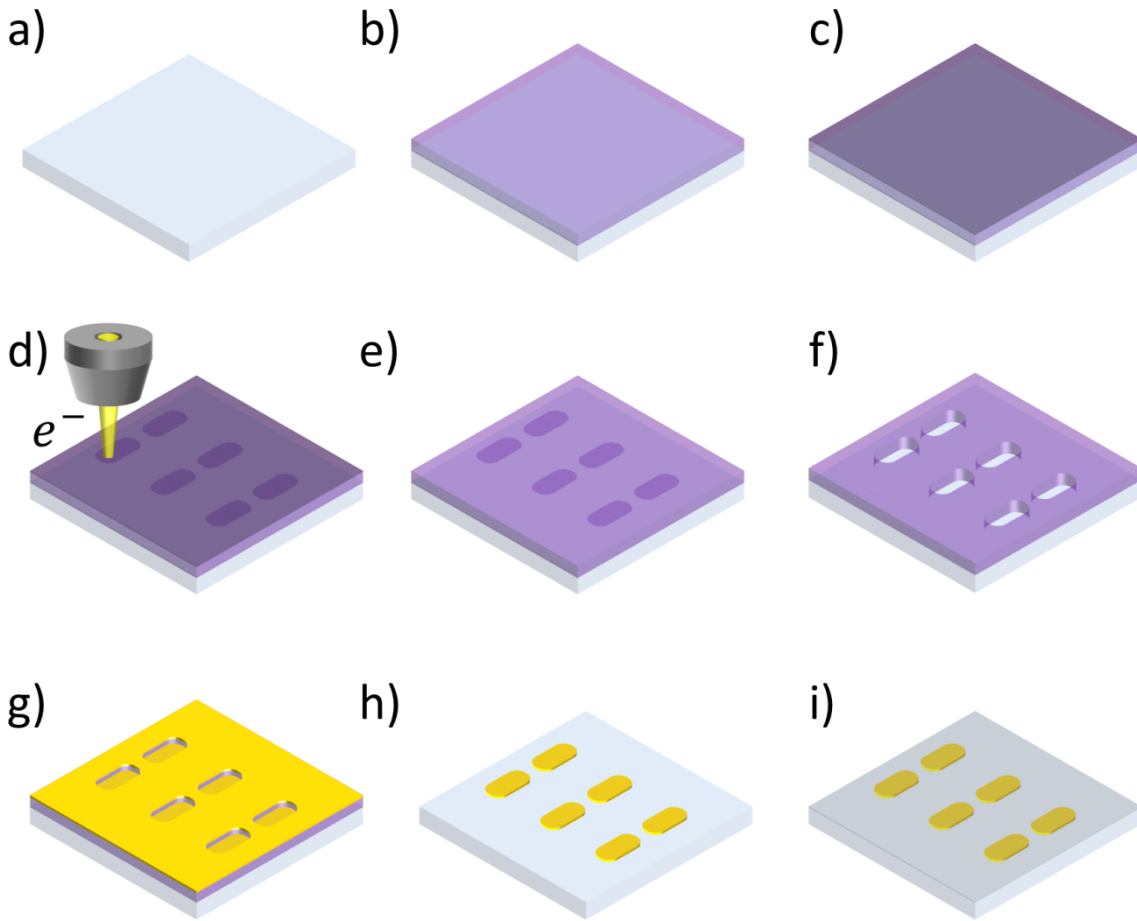


Figure 6.4 – **Schematic of sample electron beam lithography sample fabrication.** a) Cleaned substrate surface. b) Spin coated resist layer. c) Spin coated conduction layer. d) Electron beam write. e) Conduction layer removal. f) Resist development. g) Structure material deposition. h) Material lift off. i) Sample surface treatment i.e. spacer layer deposition.

After cleaning, thin 200nm layers of PMMA 950 A4 were deposited by spin coating onto the glass slides, see Figure 6.4 b). The glass slides were placed in a Laurell Technologies spin coater and covered evenly with PMMA. The slides were then spun at 3500 rpm for 60s to produce the 200nm layers. This must then be cured by baking on a hotplate for 5 minutes at 160°C.

Because PMMA is non-conductive, when writing with the electron beam charge build-up will occur at the surface. This is effectively a static charging of the surface and would cause deflecting of the electron beam, changing the shapes of the structures being written. To address this, an extremely thin conductive layer of ESPACER 300Z was spun coated onto the surface of the cured PMMA at 1500rpm for 60s. This was then baked for 60s at 100°C on a hot plate (Figure 6.4 c)). This layer dissipates any charge build up when writing.

The PMMA films then had the desired patterns written on them using a Raith e-line system (Figure 6.4 d)). It is important to get the correct dosage level for accurate fabrication. The dosage level refers to the amount of charge supplied to each point of the PMMA film to get a specific line width or area size at that point. More charge at a location will result in larger line widths and features. The optimum dosage levels were experimentally determined by writing test samples trialling many dosage levels. Samples were then analysed to find the correct dosage levels for this work using SEM.

The dosage levels used in this work were:

Line Dosage $700\mu\text{C}/\text{cm}^2$

Area Dosage: $250\mu\text{C}/\text{cm}^2$

Both line and area structures were written using 2nm step sizes.

After patterning the Espacer must be removed from the PMMA surface. Espacer is water soluble, however, if it is left for long periods on the surface it can become more difficult to remove, therefore, Espacer removal should be done as soon as possible after exposure. Submersion of the sample in DI water using tweezers is the preferred method. The sample should be gently stirred in the water for at least a minute before a secondary submersion in separate clean DI water. DI water squeeze bottles should not be used on the PMMA film, as this risks damaging or peeling the film away entirely. After Espacer removal, the clean samples (see Figure 6.4 e) are dried with a gentle nitrogen flow.

To remove the electron beam exposed PMMA from the film, it must be developed. Methyl Isobutyl Ketone (MIBK) was used as a developer for the patterns, it was mixed one part in three with IPA (IPA:MIBK – 3:1). The samples were submerged in the developer mix for 1 minute and gently stirred for the duration. After the submersion samples were immediately placed in IPA for 30s and then dried with compressed nitrogen. This step removes any MIBK from the surface to halt any more development of the pattern. It is extremely important to be as consistent as possible in the development step. Changes in MIBK concentration or development time will affect the structure size achieved for a specific electron beam exposure dosage. After development, as the PMMA used is a positive resist, the areas that were written are removed from the sample. This leaves a series of troughs whose bottom is the bare substrate, which can be seen in Figure 6.4 f).

The development step is followed by an oxygen plasma treatment using an Electron Diener Plasma Cleaner FEMTO for 7s at 40% power. After development of the PMMA there is often small amount left at the bottom of the pattern as well as rounding of the edges at the base of the walls. If these are not removed it can lead to poor adhesion of the deposited metal and problems in lift off. It can also cause the PMMA to be difficult to totally remove from the substrate, leading to left over PMMA still being attached to the surface in and around metallic structures. The oxygen plasma treatment removes a $\approx 10\text{nm}$ thick layer of PMMA, cleaning up the sample and sharpening the troughs as shown in Figure 6.5.

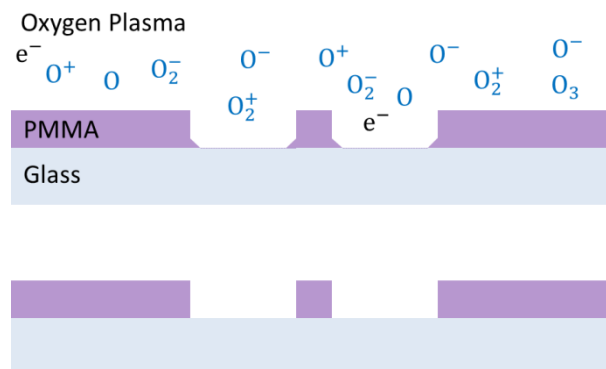


Figure 6.5 - **Example of oxygen plasma treatment.** The plasma treatment of a PMMA layer on a glass substrate is shown above using charge oxygen plasma. The PMMA shows rounding of the corners contacting the substrate. Below the treated PMMA is displayed showing sharp corners and a clean exposed substrate surface.

The samples were then coated with a layer of metal via thermal evaporation (Angstrom A-mod). Samples (s) are attached to a sample mount (SM) which is loaded into the evaporator behind a deposition shutter (Sh), as shown in Figure 6.6 a). A material crucible (C) is loaded onto the crucible mount (CM) and filled with the desired material (M) for deposition. The evaporator is then sealed and pumped down to a vacuum pressure of $\sim 1 \times 10^{-7}$ Torr. A current is then passed through the crucible, heating it and the deposition material. Once the material begins to evaporate and deposited material is detected on the growth monitor (GM), the rate is monitored and the current adjusted until the growth rate stabilised at the desired deposition value. The shutter is then opened and the material is deposited on the sample until the correct thickness is reached. The shutter is then closed and the system left to cool before venting the chamber and removing the samples. After the deposition step the sample will be covered in a film of material inside and above the troughs as seen in Figure 6.4 g).

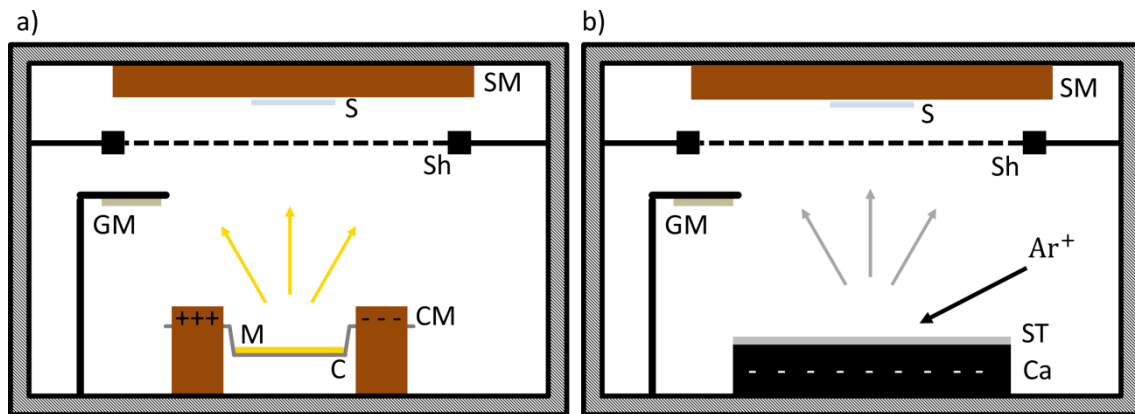


Figure 6.6 - **Schematic for material deposition techniques.** a) Evaporation deposition. Chamber under high vacuum. SM – Sample Mount, S – Sample, Sh – Deposition Shutter, GM – Growth Monitor, CM – Crucible Mount, C – Crucible, M – Material. b) Sputtering deposition. Chamber filled with Argon gas. ST – Sputtering Target Material, Ca – Cathode.

After the deposition step the material around the desired structures needs to be removed. This is done in a lift off step. In this step the PMMA below the surface material is dissolved in acetone. This is done by submerging the sample in acetone and leaving it undisturbed for several hours. For the best quality structures, it is beneficial to lift off the material with minimal agitation of the acetone and sample, as this agitation can cause the lifting off metallic film to fragment - leading to possible contamination of the

sample. Ideally the excess metal will lift off in a single film and can be removed from the acetone with tweezers. The lift off is finished by very gently spraying the substrate surface with acetone from a squeeze bottle. It is important to not directly spray the area on the sample where the structures have been written, instead acetone should be sprayed in the corners and allowed to flow across the sample. This should leave the designed structures on the substrate as shown in Figure 6.4 h).

Finally, if necessary, a dielectric spacer layer can be deposited over the metallic sample to prevent direct metal-emitter contact, leading to quenching, and to protect the structure's surfaces. The method used through this work for dielectric deposition was sputtering. Sputtering is the deposition of material on a sample through the ablation of a material source via atomic bombardment (Figure 6.6 b)). The sputtering chamber containing the sample behind a deposition barrier is pumped down to a 1×10^{-7} Torr vacuum. It is then filled with Argon to a 1×10^{-3} Torr pressure. A strong electric field is used to ionise the argon. The argon ions bombard the surface of the source (S), which is mounted on a Cathode (Ca), dislodging source material towards the sample and growth monitor. Once deposition is detected at the growth monitor the rate is stabilised at the desired value for deposition and the deposition shutter is opened. The sample is coated to the desired thickness and then the shutter is closed. The system is then left to cool and the chamber vented to remove the sample. In this work, silicon dioxide is used as the spacer layer when necessary.

After fabrication, samples must be mounted securely to the optical setup to minimize mechanical drift. Thin mechanical adhesive tape was used to attach samples (facing up) to custom fabricated sample plates as shown in Figure 6.7 a). This forms a water tight seal between the substrate and the sample plate. The sample can be removed from the sample plate by submersion in acetone which degrades the adhesive.

The sample plate was attached to a solid copper mount using four screws. Once assembled the surface of the sample can be accessed from above through the top of the copper mount as shown in Figure 6.7 b). The mount was then placed in the piezoelectric stage. It is possible to fasten the mount to the stage, however, commonly the mount

was left unfastened as contact must be made between the objective and the sample via the index matching immersion oil shown in Figure 6.7 c) which, due to the delicate nature of the sample, can easily lead to sample damage when the mount is rigidly fastened to the stage.

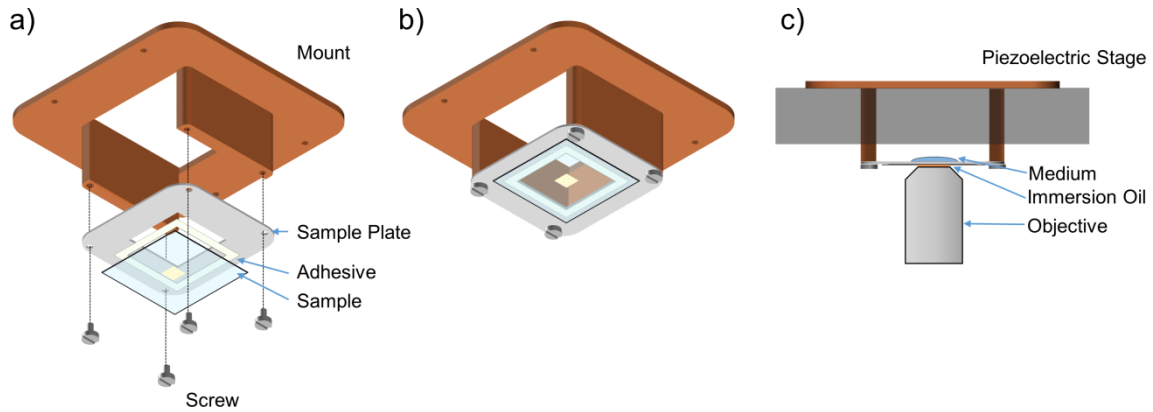


Figure 6.7 – **Schematic diagram for sample assembly and mount attachment.** a) A breakdown of the sample assembly showing sample mounting to sample plate using mechanical adhesive ($80\mu\text{m}$ thickness). b) full assembly of sample. c) Mount attached to piezoelectric stage showing objective access from below.

Once fabricated, samples must be characterised to ensure that they are of a high quality and consistency. A couple of the useful techniques for this are Dark-field microscopy and spectroscopy, discussed in the following section.

6.3 Dark-Field Microscopy and Spectroscopy

Dark-field (DF) microscopy can be an extremely effective tool for the characterisation of plasmonic antennas. Bright field and epi-illumination (reflected light) microscopy are common microscopy techniques. In these techniques, samples are directly illuminated and the transmitted (bright-field) or reflected (epi-illumination) light is collected. Both techniques suffer low contrast for small scattering objects on the sample surface, as there is a strong contribution everywhere in the image from the directly reflected/transmitted light from the substrate. In contrast, DF microscopy avoids this by illuminating the surface at an angle and collecting the high angle scattered light only. This gives the technique a “dark” background and much higher contrast between scattering objects and the background.

The DF geometry of angled illumination and high angle collection is shown in Figure 6.8 a). The reflected light that causes the bright background in epi-illumination microscopy is not collected. Figure 6.8 a) shows a “side illumination” setup for DF. This is best achieved using a long working distance air objective, with as high a numerical aperture as possible. The angle of the illumination geometry can have some effect on the spectra collected such as changing in amplitude of the detected plasmonic modes [127, 128] The collected scattered light can be imaged by placing a CCD camera at the same position as the fibre F shown in both part a) and b) of Figure 6.8. Plasmonic antenna responses can have strong dependence on illumination polarization, able to have several resonances at different frequencies with different polarizations due to their structure. It is often useful to be able to control the polarization of the illumination light, which can be done using the linear filter shown in Figure 6.8 a). This allows the selective probing of polarization sensitive resonances of plasmonic structures.

Figure 6.8 b) is a schematic of a “vertical illuminated” DF setup and makes use of a DF objective which allows angled illumination via an extra ring beam path on the outer edge of the objective and the collection of the high angle scattered light through the central column. This orientation is easier and faster to use than side illumination and the signal of the scattered light collected into the fibre is generally larger leading to a greater signal to noise ratio in the data.

The linear filter to control polarization can be placed in the illumination beam as in Figure 6.8 a) or in the collection beam as in b). For simple plasmonic structures, such as rods, this leads to effectively the same measurement for the same polarizations, as the illumination polarization is only able to produced similarly polarised scattered light from a simple antenna. There are some nano structure designs that are able to change the polarization of the scattered or transmitted light [129], however, as long as this is not the case either filter position can be used.

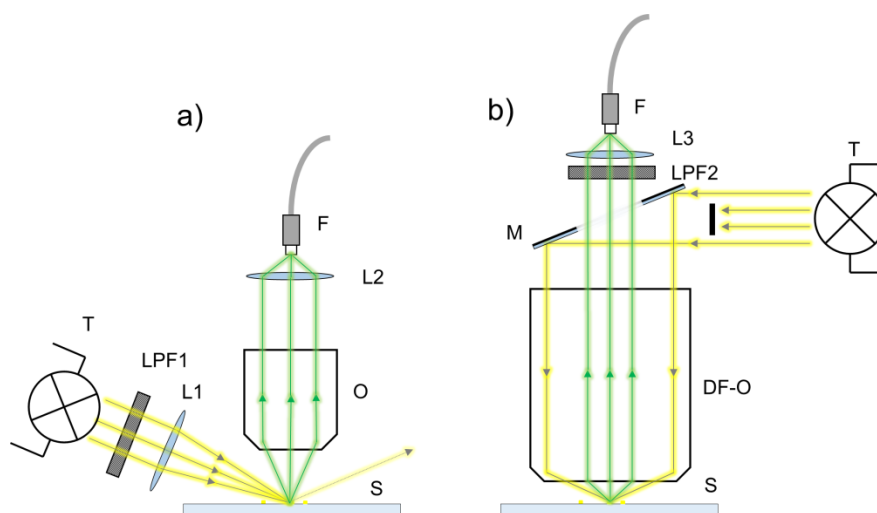


Figure 6.8 - **Schematic for Dark Field Microscopy.** F – Optical fibre, O – Objective, DF-O – Dark-field Objective, L1 L2 L3 – lenses, S – Sample, LPF1 LPF2 – Linear polarising filters, T – Tungsten filament lamp. a) Side illumination dark-field using standard long working distance objective. b) Vertical illumination dark-field using dark-field objective.

The collected scattered light is imaged onto the fibre, which acts as a pinhole. In this sense the microscope works confocally and light that is out-coupled from the fibre corresponds to a single position on the sample whose size is dictated by the numerical aperture of the system. The light level is measured using an single photon counter (SPC), shown in Figure 6.9. By scanning the position of the sample, using a piezoelectric stage, it is possible to make a map of intensity measurements forming an image of the DF scattering response of the sample. These images can be used to position the microscope's collection point over a position of interest on the sample. The fibre's out-coupled light can then be directed into a spectrometer (Princeton Instruments Acton SP2500) which, in conjunction with a CCD camera (Princeton Instruments Pixis 100), is able to take spectra of the scattered light see (Figure 6.9).

The spectra collected from the location of interest on the sample also contain a contribution in due to scattering from the sample substrate. The spectrum is further shaped by the spectrum of the illumination light and the frequency response of the optics used for collection. To achieve a meaningful measurement the data taken must be processed to remove sample background light and normalised for the optical and illumination response. This can be done using the equation:

$$I_{Antenna} = \frac{I_{sample} - I_{SubstrateBG}}{I_{Reference}} \quad (6.6)$$

Here $I_{Antenna}$ is the intensity response contribution from the antenna, I_{sample} is the collected data from the point of interest on the sample, $I_{SubstrateBG}$ is collected from a blank area on the sample (close to the subject area) and $I_{Reference}$ is a measurement made of the spectra from a reflective standard surface (Labsphere WS-1). Each measurement must be taken over the same total integration period with the dark background contribution of the CCD camera removed from the data.

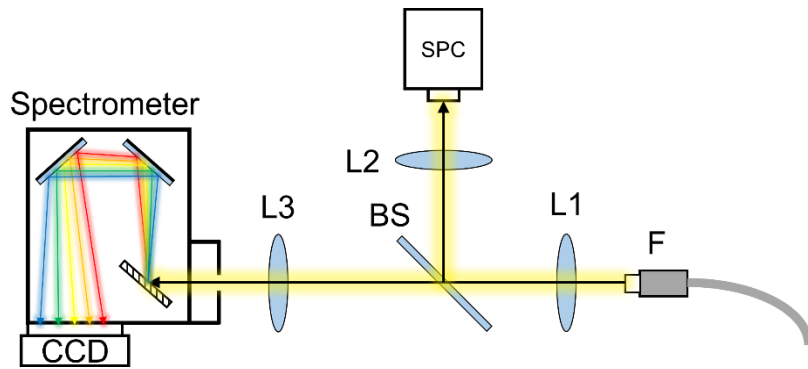


Figure 6.9 – **Photon counting and spectrometer schematic.** F – Optical fibre, L1 L2 L3– lens, BS – beam splitter, G – grating.

6.4 Fluorescent Dye Selection and Preparation

When selecting fluorescent dyes for localization based probing of plasmonic near-fields it is necessary to consider both the absorption and emission spectra of the dye to achieve the intended photonic interaction-plasmonic interaction. There are several other important optical properties to consider, such as the quantum yield, the Stoke's shift (the difference between peak absorption and peak emission wavelengths) and the excitation coefficient of the dye.

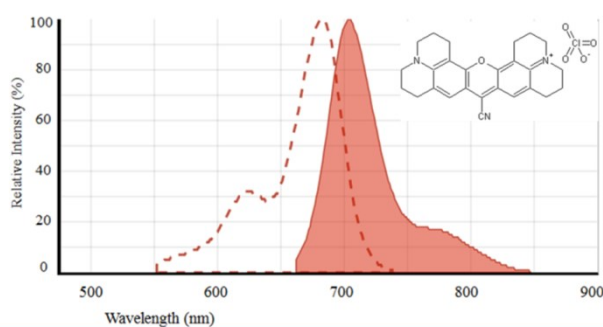
In this work three main dyes were used to investigate the near-field interactions. These were Rhodamine 800, Pacific Blue (PB) and Pacific Orange (PO). Their available relevant properties are displayed in Figure 6.10.

Rhodamine 800

$$\lambda_{ex} = 682\text{nm} \quad QY = 0.25$$

$$\lambda_{em} = 712\text{nm}$$

$$\text{Stokes Shift} = 30\text{nm}$$

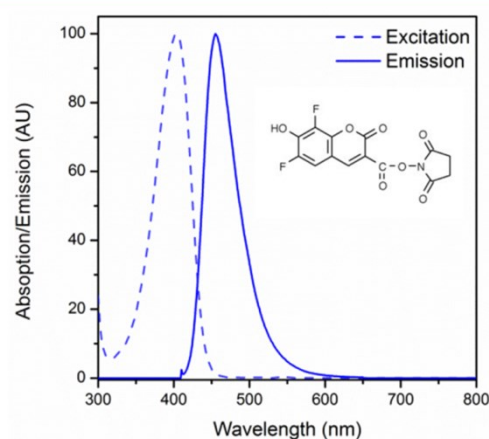


Pacific Blue – NHS ester

$$\lambda_{ex} = 416\text{nm} \quad QY = 0.75$$

$$\lambda_{em} = 451\text{nm} \quad \varepsilon = 34000\text{M}^{-1}\text{cm}^{-1}$$

$$\text{Stokes Shift} = 35\text{nm}$$



Pacific Orange – NHS ester

$$\lambda_{ex} = 404\text{nm} \quad QY \sim 0.5$$

$$\lambda_{em} = 553\text{nm} \quad \varepsilon = 25000\text{M}^{-1}\text{cm}^{-1}$$

$$\text{Stokes Shift} = 149\text{nm}$$

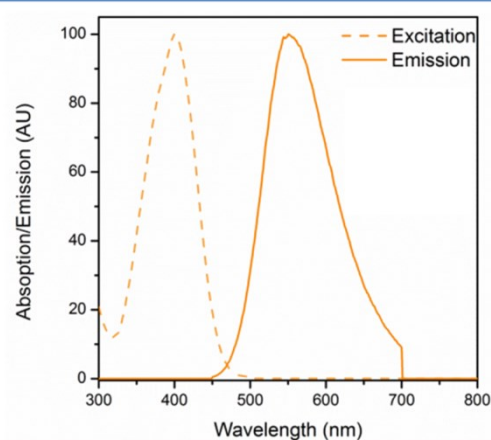


Figure 6.10 - **Properties of fluorescent dyes used for SR mapping of plasmonic antenna.** Rhodamine 800 spectra accessed from [130] and chemical structure [131], Pacific Blue and Pacific Orange spectra and chemical structure accessed from [132] and [133] respectively.

It is also important to consider the surface chemistry interaction with the dye. Some molecules will not interact well with a surface or can even be repelled entirely. As such, care must be taken to achieve the desired strength of surface interaction. As most fluorescent dyes are produced for biological research, they are commonly commercially available already functionalised with an antigen/antibody or a functional group that

allows further chemical modifications. The functional groups are used to attach molecules to other structures e.g. proteins of interest or surfaces and generally decide the overall charge of the molecule. Electrostatic interaction is a simple and effective way usually employed when functionalizing surfaces with molecules. Weak physisorption of the molecules – as desired in a PAINT style Brownian motion approach – on solid/liquid interface is achieved by this method of interaction. The overall charge of the molecules must be opposite or neutral to the surface charge to achieve the desired interaction.

At the interface between a material and a liquid solvent, a measurement of the potential of zero charge (PZC) can be made. This is a measurement of the potential that needs to be applied in order that the material surface has zero charge and it depends on the concentration and type of ions dissolved in the solution and/or its solvent or H⁺ (i.e. media). In this way, the PZC can be used to extract the surface charge density (SCD) of a material in a given media. Thus, the PZC or SCD gives an indication of which fluorescent molecule charges will interact well with the surface at the experimental conditions. Charge incompatibility with the surface can lead to low interaction rates as molecules struggle to make contact with the surface as they are being repelled by the charges at the surface.

It should be noted that using this method to select a suitable molecule can lead to surface-molecule electrostatic interactions without the need to functionalize the surface with polymers, molecules or other materials that may affect the plasmonic properties of the nanoantennas and/or leave the molecule far away from the highest near-field regions that are being explored.

The SCD for the various materials used throughout this work, measured in similar conditions to the ones used in our experiments, show that Al₂O₃ surfaces adsorb cationic species ($q \sim 1 \mu\text{M}/\text{m}^2$) [134] and that replacement of the solvent for DMSO did not alter this behaviour [135]. SiO₂ surfaces are neutrally charge in our experimental conditions ($q \sim 0 \mu\text{M}/\text{m}^2$) [134].

As the Al₂O₃/SiO₂ surfaces will be positively/neutrally charge under our experimental conditions, we selected negatively charged and neutrally charged dyes for our

fluorescent probes. Rhodamine 800 is a negatively charge probe and PB and PO are neutrally charged probes.

6.5 Sample Tracking and Stabilisation

Sample drift is a common problem in microscopy. Although most optical and microscopy-based techniques are susceptible to it, localization based microscopy, which relies on taking large sequences of images in which highly accurate sub 10nm localization will be performed, is particularly degraded by even small amounts of drift. Any variation in sample position will result in blurring and distortion of the resultant localization image. Common sources of drift and mechanical instability include thermal drift, mechanical vibrations, substrate flex, and issues related to lubricant and/or objective weight.

Thermal Drift is caused by the expansion and contraction of materials with changes in temperature. This drift has a long time frame, often leading to cycles of oscillatory motion of the sample that take hours to complete. The drift can be quite large in size leading to the complete defocusing of equipment and several microns of lateral movement over the course of an hour. This issue can be common in air conditioned labs, especially close to air vents, due to the hysteretic nature of the temperature control systems used. Other sources include any equipment near to the setup, such as halogen and filament light sources, heating their surroundings.

These thermal effects can be minimised in several ways. First it is possible to box in the microscope to insulate it from the air temperature fluctuations. The microscope should be isolated as much as possible from the sources of heat within the room, including the driving electronics for sensors or actuators which should be kept on a separate bench away from the microscope.

Mechanical Vibrations are a very common source of error in microscopy in general, with several internal and external sources being present. Internal sources of vibrations refer to equipment being used with the microscope to make measurements. Many components (laser, control electronics, etc...) require some form of cooling, often using a fan. These and other moving components can introduce mechanical vibrations to the

microscope environment. External sources of vibration are translated to the system via ground and air movement. The same air-conditioning systems that introduce heat fluctuations can also mechanically vibrate the system through air movement. Ground sources include building lifts, opening and closing doors and vehicle and pedestrian traffic.

To minimize vibrational drift a suitable optical bench should be used. The bench should be sufficiently heavy to dampen high frequency oscillations. If possible hydraulic isolators should be used with the bench which will reduce the transmission of external vibrations to the table. Sources of internal vibrations can be minimised by moving the relevant pieces of equipment off the optical bench itself when possible. Any sources of vibration that have to be on the optical bench should be on isolation mounts, however, cameras that must be stationary with respect to the microscope, such as an EMCCD camera, should be bolted firmly down to the bench and onto the microscope.

Substrate flex occurs when working with oil immersion objectives, that have short working distances, and need thin substrates. In this work 160 μ m No. 1 glass coverslips were used to match the working distance of the objective. These are extremely delicate and very susceptible to substrate flex. This can be due to thermal gradients from room environmental controls or due to local heating when under illumination. Both of these can lead to large vertical shifts in the sample position as the sample bows up and down (away and towards) the objective. Additionally, the objective being in near-contact with the sample surface via immersion oil can lead to substrate flex as the surface tension of the oil interplays with the flex of the sample.

Sample flex can be lowered by minimizing the length of unsupported substrate and using secure sample mounting materials.

Lubricant and objective weight arise simply due to the weight of objectives, and the objective carousel, being susceptible to sinking down under the force of gravity. The lubricant used inside the microscope can lead to the slow settling of microscope objectives – leading in turn to slow defocusing.

Most objective sinking problems can be avoided with correct practise when operating the focus control. Keeping the minimum number of objectives in the carousel at once will reduce the weight on the mechanism. The objective should always be brought into focus against the force of gravity. The objective must be brought into focus and allowed to settle several times for the best stability.

Other possible sources of sample drift include mechanical strain across microscope components causing sudden and large slipping; immersion oil heating and degradation which can cause extra flexing of thin glass coverslips; any addition or removal of reagents into the liquid above the sample surface, which can produce large shifts in focus and lateral position due to mechanical interaction as well as result in sudden changes in temperature of the solution, leading to increased thermal drift.

The optical setup shown in Figure 6.1 was built onto a Nikon Ti-U inverted microscope fitted with a Nikon TI-TIRF Illuminator Unit. This is centrally mounted on a heavy optical bench (Newport RS 4000) on a set of hydraulic vibrational isolators (Newport Stabilizer high performance laminar flow isolator I-2000 series). The main sources of mechanical vibration were the EMCCD fluorescence camera and the CCD tracking camera, both of which use fan assisted cooling and must be mounted on, or near, the microscope. These were bolted to the optical bench and microscope to minimize the image oscillation.

Even using best practices, it is extremely hard to fully eliminate drift over the timescales of the long imaging sequences used in this work. As such, we have designed automated drift correction system, using the piezo electric stage on which the sample is mounted, to adjust for any sample movement – maintaining focus and position.

To achieve this, a monitoring arm was built onto the microscope using a beam splitter (BS in Figure 6.1) to collect some of the scattered laser light from the sample surface. This light was imaged using a secondary camera. Note that the BS also collects the returning illumination beam that was totally internally reflected at the substrate/liquid interface. This returning beam must be blocked using a beam block as it obscures the weaker scattered light from the surface antenna.

The nanoscale plasmonic antennas studied form scattered laser light PSFs in the tracking camera as they are sub wavelength in size. It is possible to use the same MLE method that is used to localize the position of fluorescent molecules (section 4.3) on this scattered light PSF from a known reference point on the surface in order to track motion of the sample with nm accuracy [136].

Correction of xy lateral movement becomes a simple case of monitoring any such movement and moving the piezo stage appropriately to maintain the position of reference point antenna in the image field. Correcting for defocus is more complex. For common optical setups the defocus causes the PSF to widen in x and y . This can be seen in the upper part of Figure 6.11 c), where a single antenna was repeatedly localised as its position was shifted in z using the piezo stage. The widths, in x and y , of the Gaussian fitted to this PSF are σ_x and σ_y , respectively. For a circular reference point, and ignoring the cylindrical lens in Figure 5.3 a) momentarily, one expects $\sigma_x = \sigma_y$ and both broaden equally as the sample is defocused. For the case of our samples, the slight sample asymmetry leads to an offset σ_x and σ_y as the beam is defocused, as shown in the top of Figure 6.11 c).

Thus correcting for the defocus is non-trivial in a “basic” imaging system, as the defocus in the $+z$ and $-z$ direction appear the same. To do so would require either working from an initial defocus or a more complex adjustment system. To overcome this issue, a cylindrical lens (CL) is inserted along the optical path as shown in Figure 6.11 a). This lens causes an asymmetric distortion of the PSF with positive and negative defocus. Instead of symmetrically expanding for both directions of defocus, the PSF widens in x for positive defocus and widens in y for negative defocus as shown in Figure 6.11 b). This leads to two linear relationships for σ_x and σ_y with defocus, around the focus, as shown in the lower part of Figure 6.11 c). This linear relationship fails for high levels of defocus. The linear phase of this relationship can be scanned before tracking and the gradient determined giving the amount that either σ_x or σ_y will change with z movement. This can then be used to correct for any vertical sample drift – making it possible to maintain focus over extremely long scan times.

Each sample array was fabricated with a central tracking marker, normally a disk of $\sim 200\text{nm}$ in diameter, which could be used to track the position of the sample.

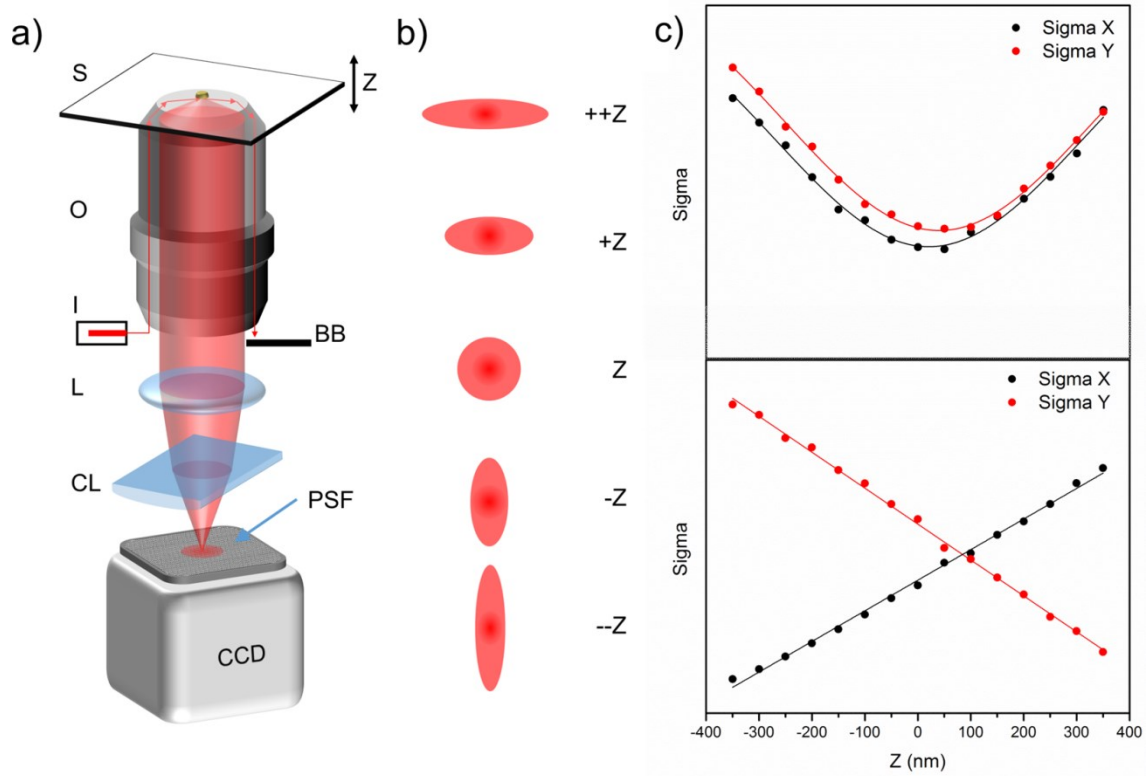


Figure 6.11 - **Schematic for sample tracking optics using cylindrical lens.** S – Sample, O – Objective, I – Illumination laser, L – lens, CL – Cylindrical Lens, BB – Beam Block. a) Illumination and collection of scattered light form a tracking antenna using a cylindrical lens and a CCD camera. b) Schematic of PSF variation for cylindrical lens focused light as focus is adjusted. c) Measured σ_x and σ_y variation for MLEM Gaussian fitted PSFs for common lens focused (TOP) and cylindrical lens focused (BOTTOM) light.

A direct correction of the any change in position as it is observed, known as a proportional correction system, is an obvious choice to adjust for any drift. This, in its simple form, involves the CCD camera taking an image, localizing the tracking antenna, calculating a change in the current position Δxyz with respect to the original position and moving the piezo electric stage $-\Delta xyz$ to correct for the drift. This approach is unsuitable, however, as we are attempting to correct to position of the system for a slow varying drift to a tolerance that is close to the error not only of the PSF localization but also of the piezo electric stage's movement error. Any under or over estimation in the localization or the expected stage movement can lead to under or overcompensation of

drift. This could lead to a rapid vibrational movement of the stage or more often lead to a growing instability that would, if not restricted, drive the stage passed its limits.

As such, a proportional integral differential (PID) control loop was implemented to stabilise the drift correction. This technique uses three weighted contributions; from the most recently read difference between position and original position, from the previous differences between read positions and original position and a prediction of future positions using the previous readings to estimate a gradient of change. These contributions correspond to the proportional, integral and differential components of the control loop, respectively. These contributions are used to calculate a movement function U which is actuated by the piezo electric stage to stabilise any drift. Equation (6.7) describes the calculation of the movement function U .

$$U = k_p e(t) + k_i \int e(t) dt + k_d \frac{de(t)}{dt} \quad (6.7)$$

Where k_p k_i k_d are the weights for the proportional, integral and differential contributions, respectively, e is the error in position ($= \Delta xyz$) and t is the time (which refers to the frame number). Because this PID function is being applied to a system whose current position is being slowly perturbed by an external force, the integral and differential terms become a windowed running summation and a windowed linear fit of the data. This windowing is necessary, as data points from further in the past do not describe the current external force, which can change over time, and so must have their influence removed from the correction process. Equation (6.7) can be rewritten as:

$$U = k_p e(t) + k_i \sum_{t-N}^t e(t) + k_d \left[\frac{de(t)}{dt} \right]_{t-N}^t \quad (6.8)$$

Where N is the number of previous frames analysed to calculate the current movement function. This control approach was written using LabVIEW, which interfaced the input from the CCD camera (QICAM 12-bit Color Fast 1394) which was used for monitoring the tracking antenna, to the signal controller box (Nanonis by Specs) which adjusted the position of the piezo electric stage (PI 733.xyz). The barebones of the control loop are shown in Figure 6.12.

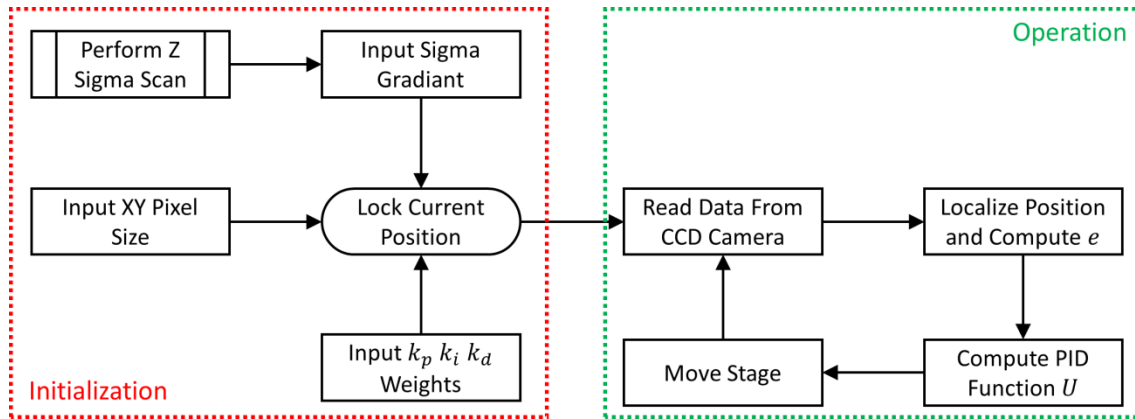


Figure 6.12 - **Control loop for sample stabilisation.** Data take from the tracking CCD camera, localized with MLE method fitted Gaussian and movement controlled with PID control loop.

The input parameters necessary for the correct functioning of the control loop are a pixel scale, a sigma vs z variation gradient and PID weights. The pixel scale can be determined by measuring the line width between several antennas in the array for which the separation is known. The sigma vs z gradient can be achieved by a linear fit of the reference point PSF broadening during a z scan, as shown in the lower part of Figure 6.11 c).

To monitor the drift variation with and without tracking, and hence to validate our drift correction program, scattered laser light was collected from the EMCCD camera by removing the dichroic and fluorescence emission filters and replacing it with a 50/50 beam splitter as shown in Figure 6.13 a). This allows the localization of the same antenna on both cameras. When tracking is enabled, the CCD camera is used to track and adjust position while the EMCCD is monitoring how successful this process is. Figure 6.13 b) shows the deviation of the localization position of the tracking antenna with respect to the initial position in x and y . The images to track have the same integration time used for fluorescence monitoring. The sample was set up and brought into focus with liquid on the sample surface, illuminated, then left to warm up and settle before being brought back into focus to monitor the drift. Over the 17-minute run, the total lateral drift is $\sim 37\text{nm}$ and mostly appears to be due to thermal drift. With the tracking activated, the xy variation is kept within $\pm 10\text{nm}$ from the origin at all times, as shown in Figure 6.13 c). There is some driven oscillation around the centre which could be reduced with

better optimisation of PID weights and movement thresholds. It should be noted that in both x and y , with tracking on or off, there appears to be variation around a central position of around $\pm 3\text{nm}$. Due to the extremely high photon number collected for scattered laser light in a short integration time ($\sim 6 \times 10^5$ photons) and the comparatively low background light levels (~ 1.1 photons/pixel) giving an high signal to noise ratio, the accuracy of the localization, calculated using the variance in Equation (4.8), is $\pm 0.676\text{nm}$. This is very precise when compared with single fluorescent molecules measurements and is much smaller than the detected variation which is likely due instead to the mechanical vibration from the high speed fans used for cooling the CCD and EMCCD cameras. This latent vibration is unavoidable without switching cooling methods for the EMCCD camera. This vibration also makes the correction feedback loop more challenging as the desired stability of the microscope is close to the “fuzziness” of the tracking antenna position.

The algorithm has limitations to the size of perturbation it is able to correct for. Therefore the tracking algorithm must be enabled only after all reagents have been added to the sample surface and the sample brought into focus. Any addition of new reagents or large knocks to the optical bench will cause the tracking system to fail.

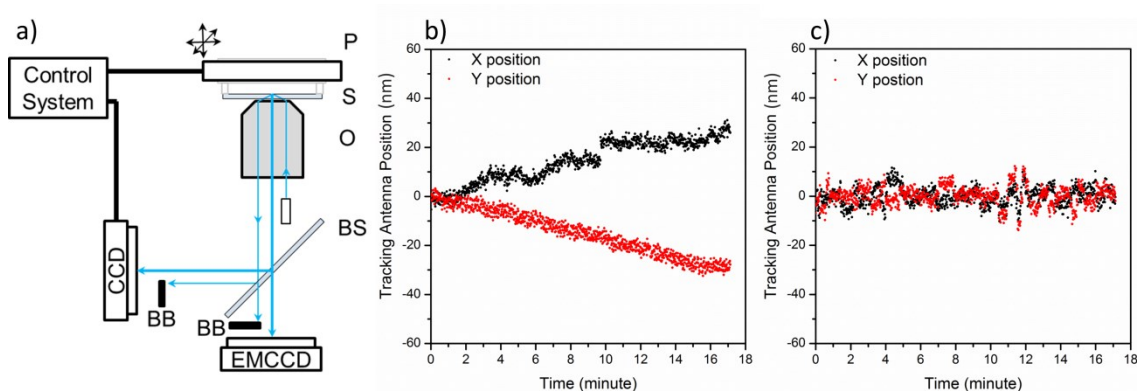


Figure 6.13 - **Sample stability test with/without tracking.** a) Schematic of scattered laser light collection in monitoring EMCCD and tracking CCD camera where P – Piezoelectric stage, S – sample, O – Objective, BS – Beam Splitter, BB – Beam block. b) Localization results from EMCCD monitoring camera for tracking antenna with tracking off. c) Localization results from EMCCD monitoring camera for tracking antenna with tracking on.

7. Results

We now examine the results from SR mapping of three different types of plasmonic structures in the following sections. Each section considers details of the sample fabrication, characterisation, methodology (outlining any specific experimental optical setup and dye usage used for a particular sample), and results and discussion for a given structure.

7.1 Probing the Near-Field of Rough Gold Films

The 2011 Cang *et al.* paper that spawned the mapping of plasmonic near-fields using randomly absorbed probes looked at hotspots generated on rough aluminium films illuminated with a 532nm laser using Chromeo 546 dye as a probe molecule [89]. Here, we study similar hotspots generated by rough gold films.

7.1.1 Sample Fabrication

Samples were made by depositing of 25nm layers of gold (Testbourne Ltd., Au purity 99.99%) onto VWR No.1 coverslips. Coverslips were cleaned with a standard cleaning procedure (section 6.2) and deposition was performed via thermal evaporation (Angstrom A-mod) at a deposition rate of $2\text{\AA}/\text{s}$. This gold layer was then scoured in places with a pair of tweezers to add rough areas with sharp features capable of producing near-field enhancements when illuminated. The marks also have the benefit of making the sample surface easy to find and bring into focus without using surface markings that can contaminate the sample.

After the sample is scoured, a 4nm layer of SiO_2 is deposited on the surface of the gold and exposed glass via sputtering at a rate of $0.2\text{\AA}/\text{s}$. This layer acts as a spacer layer to prevent the energy transfer quenching of fluorescent molecules which occurs close to the surface of metallic layers [50].

7.1.2 Methodology

The sample was mounted onto a sample plate and fixed onto the microscope as shown in section 6.2. It was illuminated using a 640nm laser (Picoquant CW LDH-D series) coupled through a fibre (Thorlabs multimode) and launched into the Ti-TIRF illuminator.

The probe molecule used was Rhodamine 800, details of which were provided in section 6.4. The filter set included a laser clean up filter centred at $642 \pm 10\text{nm}$ (Chroma XET642/20x), a dichroic filter whose transition is at 647nm (Chroma ZT647rdc), and two emission filters a long pass filter at 665nm (Chroma ET665lp), and a band pass filter centred at $705 \pm 72\text{nm}$ (Chroma ET705/72m). $100\mu\text{L}$ of Deionised (DI) water was added to the surface and the sample brought into focus using the 100x TIRF oil objective. The sample /system were then left to settle. After half an hour $20\mu\text{L}$ of a 10nM DI water solution of Rhodamine 800 was added to the sample surface and a sequence of 8000 images was acquired with an exposure time of $100\text{ms}/\text{image}$, giving a run length of $\sim 17\text{minutes}$ (including the 30ms dead time between each image). In this sequence there were several locations upon the sample which exhibited repeated fluorescent interactions at the same apparent location on the surface. These corresponded to single fluorescent molecules adsorbing to the surface of the SiO_2 in the vicinity of a hotspot, leading to an increase in fluorescence brightness at these locations. These hotspot interactions were localized to form sequences of spatial xy coordinates with corresponding fluorescent intensities. A representative hotspot is shown in Figure 7.1 – the details of which are discussed in the following section.

7.1.3 Results and Discussion

After the acquisition sequence was localized into a string of xy coordinates and intensities they were normalised using the fluorescence intensities collected for individual unenhanced Rhodamine 800 molecules interacting on a poly-l-lysine film. The relative integration time and illumination intensities were taken into account. This data was then plotted as a 3D scatter graph shown in Figure 7.1 a). This gives an idea of the overall shape of the hotspot which shows similar behaviour to the AI hotspots seen in the Cang *et al.* paper. In the scatter graph each point relates to a single fluorescent molecule interaction with the hotspot from the surface of the SiO_2 layer which formed a fluorescent PSF in the image field which was then localized. The distribution shows slight asymmetry in the distribution in the x direction, however, it is difficult to see in the 3D scatter plot.

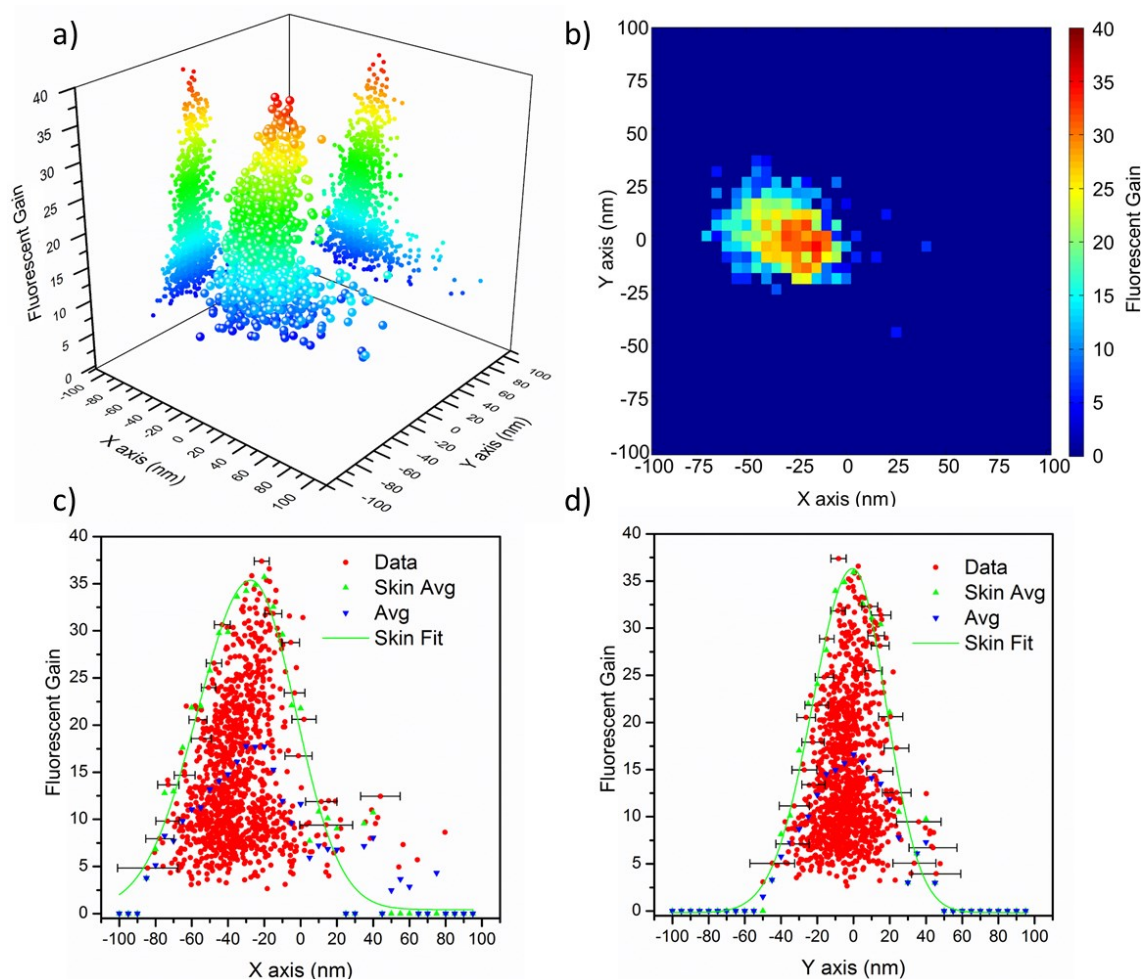


Figure 7.1 – **Localization data for rough Au film hotspot** a) 3D scatter plot of the localization results for a single Au hotspot. b) Skin average fluorescent enhancement distribution for Au film hotspot with 5nm pixels. c) Localization data viewed only in the x axis. Red points are localization data, blue points are average gain for that 5nm x position, green points are the skin average gain for that 5nm position. d) Localization data view only in the y axis. Red points are localization data, blue points are average gain for that 5nm y position, green points are the skin average gain for that 5nm position.

As previously discussed in section 3.2, due to the random orientation of the adsorbed fluorescent molecules' on the collected fluorescence, only the molecules which were well aligned with the illumination field, and in an optimum collection orientation at each position of the surface, make up the envelope of the localization distribution. This means that the information of the near-field distribution strength is contained in the envelope. This maximum positional fluorescence corresponds to a well aligned molecule (with field and objective collection), thus the orientation effect is removed when considering the envelope. To better display the envelope a "skin average" was taken of the data. The

skin average creates a square array of bins of a set size (in this case 5x5nm) and takes the average of the brightest 3 events to give an indication of the fluorescence gain at the pixel location. This skin average data is then plotted as a colour xy plot shown in Figure 7.1 b). The asymmetry in x can be seen more clearly in this figure, however, it is difficult to relate this asymmetry to any feature of the system i.e. whether this arises due to illumination direction or the shape of the gold structure as the structure, is unknown. Because there are land marks made on the film samples finding the area and knowing which specific structure in that area generated the plasmon would be an unfeasible task.

It is common in the literature to plot colour distributions that are calculations of the total average fluorescence at each pixel location instead of a skin average [137, 138]. The total average is an unsuitable approach which can be seen in the x projection scatter plot shown in Figure 7.1 c). This plot is a side view of the fluorescent gain variation in x where each red point is a localization. The points in green and blue are the skin average and total average, respectively. It can be seen that the skin average closely resembles the fluorescent gain envelope whereas the total average provides a poor representation of this envelope. It is possible that if the total number of interactions collected is particularly low that the total average would give a smoother result, as it makes use of all available data points. However, if the number of localizations is low enough for this to be the case, the envelope will not have been built up significantly, thus the data will yield poor results regardless.

Figure 7.1 d) displays the y projection of the localized data (red points) and the corresponding skin and total averages in green and blue, respectively. It shows the same low estimation made by the total average as for the x projection. The y projection shows a much more symmetric variation of localization field when compared to the asymmetric x variation.

Both Figure 7.1 c) and d) have error bars on a selection of the localization points at the envelope edge to indicate the level of precision that these localizations were made to. This error was calculated using the variance calculation (equation (4.8)). The envelope

that is made up of the brightest observed fluorescent molecules at each location, it should be noted that these are also the molecules with the highest accuracy in their localized position, as a larger number of collected photons gives a more accurate Gaussian fit. This relationship can also be seen in Figure 7.2. The peak fluorescence in these rough Au hotspots and for others like it corresponds to a ~ 35 fold increase in collected fluorescence, with a FWHM for Figure 7.1 of the skin fit in x and y of 62nm and 43nm respectively. This and others like it seem to indicate a larger hotspot size for this rough Au than the hotspot size shown in the Cang *et al.* paper for rough Al films which were as small as ~ 15 nm.

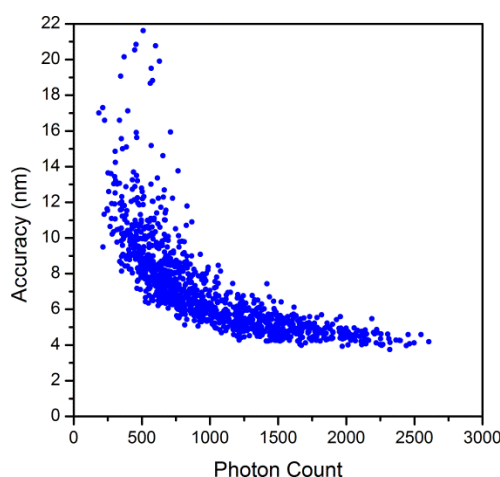


Figure 7.2 - **Scatter graph of accuracy of localization plotted with photons collected.** With increasing photon counts the accuracy of the localization id improved due to better quality fits of the Gaussian to the PSF distribution.

7.2 Probing the Near-Field of Gold Plasmonic Structures

Probing the near-field of rough films served to demonstrate the general applicability technique to mapping a plasmonic hotspot, however, it is not easy to determine and model the underlying structure of the film producing the hotspot, making it difficult to verify that the results match those predicted by theory and simulation. We were unable to take any spectra or SEM of the active locations on the Au films. Thus we progressed to using electron beam lithographically fabricated nano-antennas. This allows for precise and large area control over the geometry and distribution of hotspots for our sample. It also makes it possible to match features in the localization distributions to specific details and features in the designed antenna geometries to truly validate the

technique. Gold was chosen for the preliminary structure studies as it is a relatively easy material to work with, however, as will be discussed, in subsequent sections we progress away from gold.

7.2.1 Sample Fabrication

Antenna patterns were designed in square arrays with each antenna spaced from its neighbours by $4\mu\text{m}$. This was done to stop any plasmonic interaction between each antenna and its neighbour but also to allow multiple similar antenna to be observed in each image run. Simple nanorod dimers were chosen due to the strong fluorescence enhancement that can be produced in the gap between rods [139]. Arrays were fabricated with large corner markers to allow them to be easily found under the microscope and correlated later on with SEM images.

PMMA structure patterns were written on cleaned VWR No.1 cover slips as documented in the fabrication section 6.2. They were then developed using a MIBK:IPA solution and treated with an oxygen plasma to sharpen the pattern and improve adhesion. 25nm of gold (Testbourne Ltd., Au 99.99% pure) was deposited onto the surface at a growth rate of $2\text{ \AA/s}$ followed by a lift off step in acetone. The bare sample was then coated with a 5nm spacer layer which was deposited via sputtering at a growth rate of $0.2\text{ \AA/s}$. This thickness was chosen to attempt to prevent close range fluorescent quenching of fluorescent probes.

7.2.2 Characterization

The geometry of a dimer bar antenna is shown in Figure 7.3 a). The antennas are formed of two symmetrical Au bars of length L , width W , height H , spaced by a gap G and coated in a deposited spacer layer of SiO_2 with thickness D which is shown in the cut out. The long and short axes are indicated by the blue and red arrows, respectively. This geometry is replicated in the simulations of the structure.

After fabrication, samples were mounted on the DF microscope (shown in 6.3) where images and spectra were taken compared with simulated antenna responses. A DF image an array of gold bar dimers is shown in Figure 7.3 b) ($L=120\text{nm}$, $W=50\text{nm}$, $G=40\text{nm}$ and $H=25\text{nm}$ with a SiO_2 layer D of 5nm). By observing this DF microscopy image of the

array, it can be seen that the fabrication was quite consistent between antennas and that only a few of the antennas were damaged or removed in the process. With the DF microscopy setup in a vertical illumination geometry, spectra of individual bar dimers were taken using a linear filter before the collection fibre to select the long and short axis resonances. An example spectrum is shown in Figure 7.3 c), where the red dots corresponds to a spectrum of scattered light collected from the short axis resonance and the blue dots corresponds to scattered light collected from the long axis resonance. It is the long axis resonance that is of the most interest to this work, as this resonance excites across the antenna gap. This is the location where the majority of the fluorescence enhancement occurs [46].

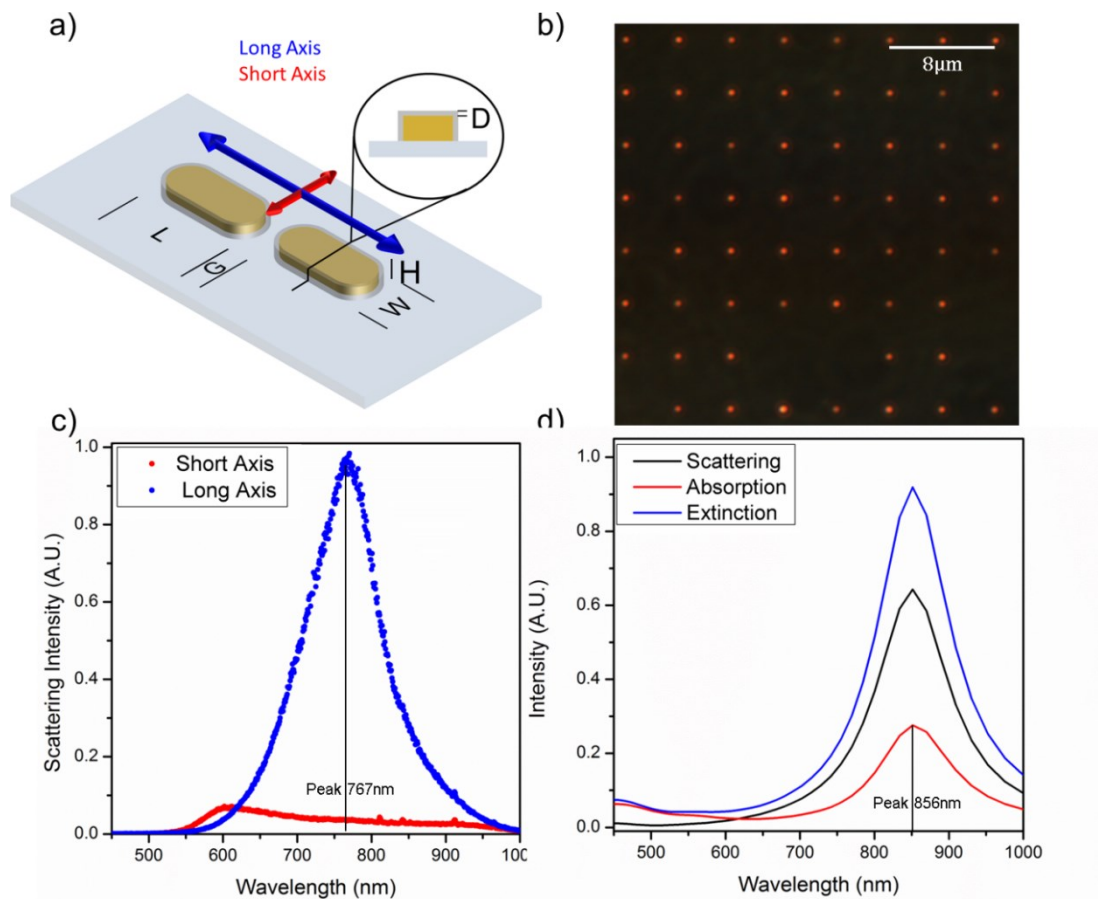


Figure 7.3 - Dimer sample geometry and plasmonic response. a) Dimer geometry where L is the length of both gold bar, G is the gap between the gold bar, W is the width of the gold bar, H is the height of the gold bar and D is the thickness of the deposited spacer layer of SiO₂. The directions of the long and short axis polarizations are labelled by the blue and red arrows, respectively. b) Dark field spectra of a L 120nm, W 50nm, H 25nm, G 40nm and D 5nm (nominally) gold bar dimer for light polarized along the long (blue) and short (red) axes. c) Simulated absorption, scattering and extinction response for the antenna dimensions in b), where blue is the extinction, black the absorption and red the scattering.

Simulations of an antenna were performed using Lumerical FDTD. By illuminating a model of the gold antenna (optical constants from Johnson and Christy) on a glass substrate (optical constants Palik), with polarization oriented along the long axis of the antenna, power in and out of the simulation space could be monitored, allowing for a calculation of the light which is scattered and absorbed by the structure. This can then be used to calculate the extinction spectrum for the structure. Simulating the antenna geometry of Figure 7.3 a) including the 5nm coating of SiO₂, (optical constants from Palik) results in the spectra of scattering, absorption, and extinction spectra (red, black and blue, respectively) shown in Figure 7.3 d).

The peak scattering response of the simulated antenna is at a wavelength of 856nm. This is red shifted by 89nm from the dark field measured response of the long axis resonance of the antenna. This is likely due to the lower quality of SiO₂ film and gold achieved in fabrication when compared to simulation although the full reason for this shift is unknown. Small gaps and pockets in the film that occur during fabrication can lower the effective refractive index of the film. This lower refractive index can affect the peak scattering wavelength of the antenna, as plasmonic antenna are very sensitive to changes in refractive index of their surroundings. This can be seen in Figure 7.4 b) which shows the change in the position of the peak scattering response of a similar bar dimer antenna system as the index of the spacer layer is adjusted from a refractive index of 1 to 1.5. An increased refractive index leads to a red shifting of the plasmonic resonance's peak scattering wavelength.

This red shift due to an increase in the surrounding medium's refractive index must also be considered for the addition of the liquid containing the fluorescence probes, which is water in this case (refractive index ≈ 1.33). The plasmonic resonances of our structures will be red shifted during the localization experiments due to this liquid when compared to the DF measurement taken in air (refractive index ≈ 1). This is illustrated in Figure 7.4 a), which shows simulated scattering responses for the same gold bar on a glass substrate in air (black), on a glass substrate with a 5nm layer SiO₂ coating (red) and then including a water medium (blue).

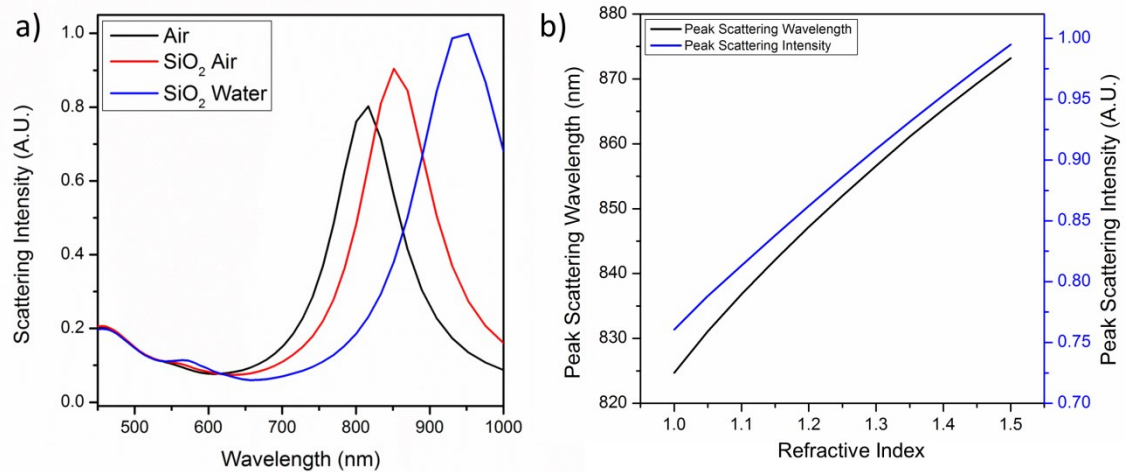


Figure 7.4 – **Effector of the surrounding medium refractive index on the scattering response of dimer antenna.** a) Simulated scattering response for a L 120nm, W 50nm, H 25nm, G 40nm and D 5nm gold bar dimer in different media and with/without spacer layers. The black line is bare gold bars on glass in air, the red is gold coated in SiO₂ on glass in air and the blue line is gold coated in SiO₂ in a liquid medium. b) Plot of how the refractive index of the 5nm spacer layer effects the peak scattering wavelength and intensity of the antenna.

To assess the quality of electron beam fabrication, SEM images were taken of the sample. To do so the sample was coated with a thin layer of ESPACER via spin coating to prevent charge build-up on the surface and improve image quality. Multiple images of antennas that produced the DF spectra in Figure 7.3 c) were taken and are shown in Figure 7.5 with measurements of their dimensions. The antennas were observed to be ~10nm larger in each dimension than the design values, corresponding to the 5nm layer of SiO₂ deposited over the gold. The layer of SiO₂ causes some of the fuzzy shadows around the edges of the antenna due to the comparatively low atomic weight of the SiO₂ with respect to the gold. Figure 7.5 (3) is an SEM image of the exact antenna whose DF spectra were presented in Figure 7.3 c).

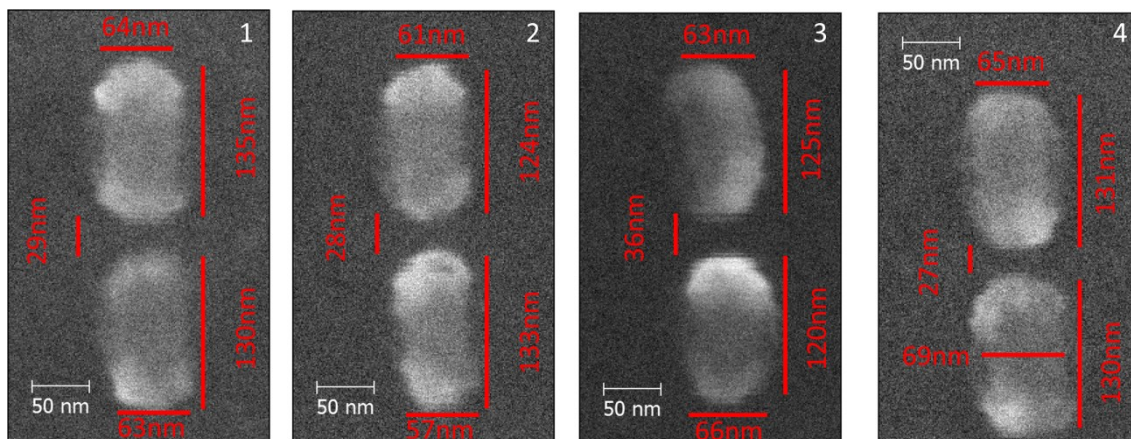


Figure 7.5 - **Scanning electron microscopy images of Au dimer antennas.** Images of a L 120 W50 G40 Au antennas coated with 5nm of SiO₂

Following the SEM results and the simulation of the antenna's scattering, absorption and extinction spectra, a simulation of the electromagnetic field gain squared ($|E|^2/|E_0|^2$) was produced for an L 120nm G 30nm H 25nm antenna coated in a 5nm layer of SiO₂ illuminated by 640nm light. The field was averaged over several xy plane slices along the z axis over the exposed surface of the antenna. The resultant distribution is shown in Figure 7.6. This simulation shows a 2.5 fold increase in the EM field strength in the gap between bars. This is quite low, but is not un-expected due to the off resonance nature of the illumination wavelength. The simulation also shows field enhancement along the upper and lower edges of the bars. However, as the excitation laser is off resonance with the antenna, it is expected that excitation enhancement is not the primary contributor to enhancement in these antennas. Rather, because the dye emission peak is at 712nm, closer to the long axis resonance peak, and we would expect to have mostly emission, with this enhancement originating primarily in the antenna gap.

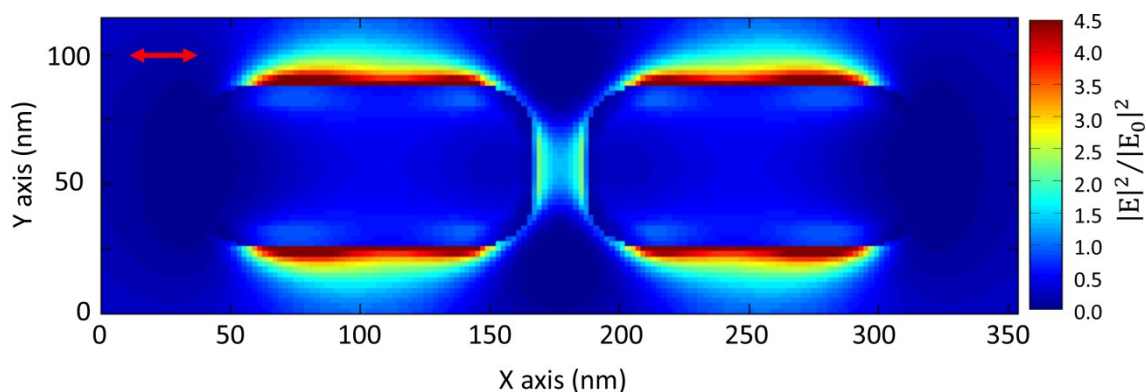


Figure 7.6 – **Simulated electromagnetic field enhancement for an Au dimer for 640nm illumination.** Gold dimer of dimensions L120 W50 G30nm on a glass surface with a 5nm coating of SiO₂ over both the glass substrate and gold. The simulation was done with light polarized along the direction of the red arrow in the figure.

7.2.3 Method

The sample was mounted to the microscope, using the same optics as documented in section 7.1.2. After adding 100μL of DI water to the surface and bringing the sample into focus, the sample was left to settle for half an hour. Following this, 20μL of a 10nM of Rhodamine 800 was added to the surface. The sample was then illuminated with 640nm laser (Picoquant CW LDH-D series) in TIR with the polarization aligned with the long axis of the gold antenna. The light was polarized along the surface of the substrate it couple as much light into the lateral optical modes of the antenna as possible. A sequence of 100ms exposure images was taken using the EMCCD camera for 4000 frames with 30ms dead time between each image, giving a run time of ~9min. This data was then processed and the relevant PSFs were localized to produce a data set of localization positions with corresponding fluorescence intensities. These were then processed into skin average localization field distributions.

7.2.4 Results and Discussion

Results from the Au bar dimer antenna consistently show single patches of enhancement and events for each antenna system. These patches correspond to the antenna gaps. This conclusion was made after attempting perpendicular polarisation illumination of the dimer antenna (short axis illumination) although this places us on resonance with the short axis (see Figure 7.3 c)) little to no fluorescence was observed

around the antenna. This indicates that the gap plays an important role in the excitation of fluorescence via emission enhancement.

The commonly observed distribution and fluorescence gains achieved by the Au bar dimers is shown in Figure 7.7. This gives an example of two different L 120nm, W 50nm, H 25nm and G 30nm antenna, as shown in Figure 7.6 a), that give a good indication of the largest and smallest localization distributions mapped Figure 7.7 b) and c) respectively. Each pixel overlaid onto the antenna has a dimension of 5nm × 5nm. Fluorescent values probed were ~18-22 times greater than the background emission. In contrast with these ‘common’ distributions, Figure 7.8 is an example of an unusual antenna, imaged with SEM in Figure 7.5 (3). This antenna shows asymmetry in the distribution of gold in the upper bar. Consequently, it seems this has led to double the fluorescence gain and modification of the gain profile from the circular localization distribution seen for other bar dimers. This new distribution is more asymmetric. This is likely to be due to an increased focusing effect within the antenna’s gap leading to a smaller but more intense field distribution.

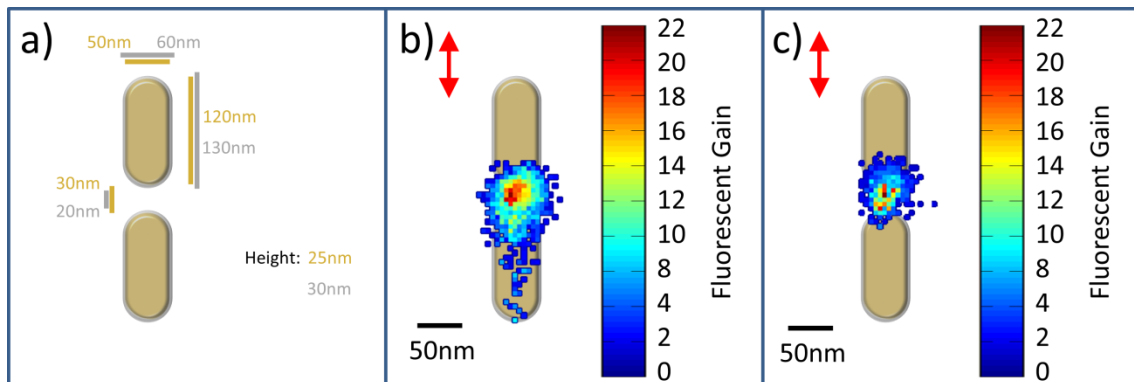


Figure 7.7 - **Localization results for L=120nm, W=50nm, H=25nm and G=30nm bar dimer antenna with 5nm spacer layer of SiO₂.** a) A schematic of the designed geometry of the antenna probed in this figure. b) and c) examples of typical localization fields for gold antenna showing the areal distribution range of enhancements seen. Samples were illuminated with 640nm linearly polarized light in TIR with the polarization orientation as indicated by the red arrows. Pixel size is 5x5nm with colour of the pixel being the average of the top 3 fluorescent gain values occurring in the pixel.

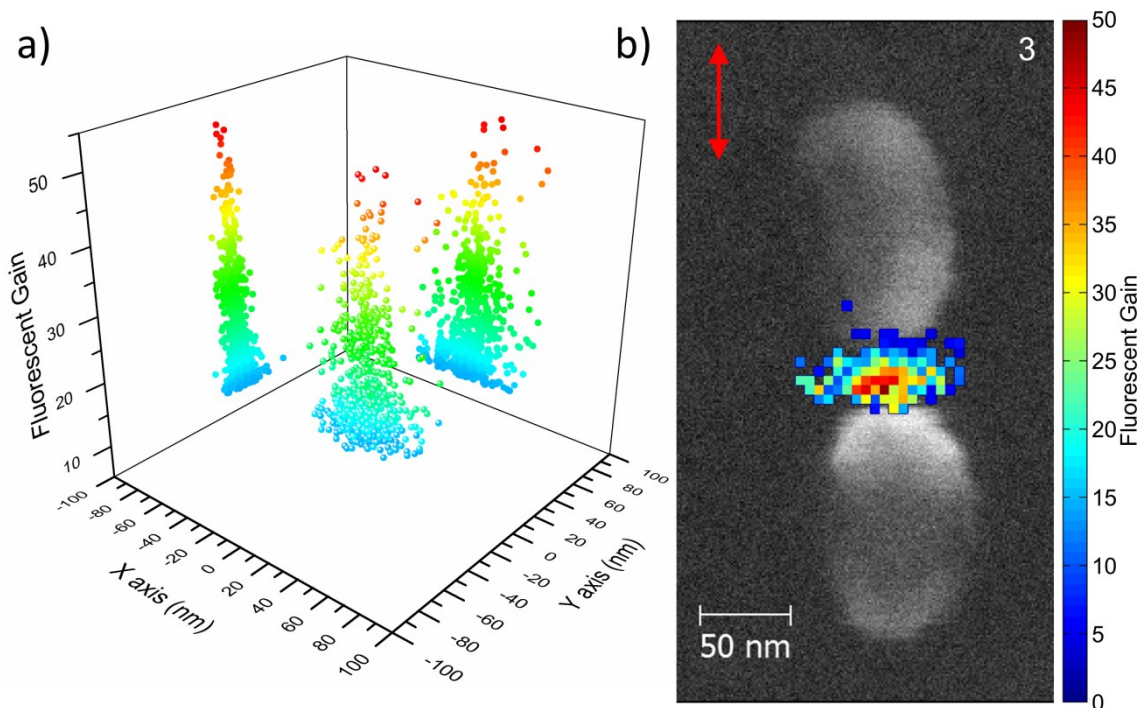


Figure 7.8 - **Localization results for L 120, W 50, H 25 and G 40 bar dimer antenna with 5nm spacer layer of SiO₂.** a) shows a 3D scatter plot of the localization results attained for antenna 3 shown in Figure 7.5. b) shows a skin average map of the localization field, to scale, overlaid in the gap of the an SEM image of the antenna being mapped. The sample was illuminated with 640nm linearly polarized light in TIR with the polarization orientation as indicated by the red arrows. Pixel size is 5x5nm with colour of the pixel being the average of the top 3 fluorescent gain values occurring in the pixel.

7.3 Probing the Near-Field Aluminium Dual Dimers

To more closely match the long axis antenna wavelength resonance to the 640nm illumination wavelength, the dimers had to be redesigned - specifically accounting for the shift in positions as a result of the dye medium changing the local dielectric environment. Achieving a 640nm resonance across a gap using gold requires very small scale structures and, with the added difficult of the red shift due to the water medium, it was determined that changing the material would be the best option. By changing the antenna material from gold to aluminium, it was, in fact, possible to have resonances at 640nm with very little modification of the antenna geometries and scales. Additionally, aluminium forms a natural aluminium oxide layer at its surface with a thickness of ~5nm [115, 140], depending on growth conditions. This layer naturally acts as a spacer layer, similar in function to the SiO₂ layer used previously.

7.3.1 Sample Fabrication

An antenna array design similar to that used in section 7.2.1 was used for the Al antenna design, however, the distance between each antenna and its nearest neighbour was reduced to $3\mu\text{m}$ to enable the observation of an increased number of antennas per image run. Initial fabrication attempts using the simple single layer 950 PMMA were plagued with poor Al adhesion issues, causing most antennas to be lost during the lift off step. Several approaches were attempted to increase adhesion between Al and the glass substrates. These included adding a chromium adhesion layer (2nm thick), trialling various self-assembled monolayers (SAM) on the glass, and using a multi-layer PMMA deposition process to reduce contact between the Al and the PMMA mask.

The SAM layers, although dramatically improving the survival rate of antenna during the lift off process, failed to secure the Al to the glass during experimental attempts. Once in solution the charged dye would cause the Al films and antennas to detach from the surface of the glass and lift off into the water medium.

Chromium layers led to a large red shift of the plasmonic resonances and would require an extra sputtering deposition step to add a new spacer layer to the surface.

Twin layer PMMA deposition proved to be a successful technique and, when combined with a change in the evaporation source position in the deposition chamber from a side to a central position (leading to growth in a more perpendicular direction to the sample surface), led to highly consistent lift off processes with low antenna loss rates. The initial off-centre aluminium evaporator position caused a build-up of aluminium against the side wall of the PMMA, masks leading to difficulties during the lift off step (see Figure 7.9 a). Centralising the position greatly reduced this effect and improved lift off Figure 7.9 b).

The twin layer PMMA mask used two types of PMMA, the original 950 and also a dilution of PMMA 495 in anisole (20ml of PMMA 495 to 6ml of anisole). First a layer of the PMMA 495 was spin coat deposited onto the surface of a clean glass slide for 60s at 5000rpm followed by a hot plate bake at 180°C for 90s. It was then left to cool for 10-15 minutes. A layer of PMMA 950 was then spin coated on top and baked using the standard

procedure documented in section 6.2. An E-spacer layer is then added on top of this, as usual. The PMMA is then patterned as normal, using the same dosage settings during the e-beam step, and then developed and oxygen treated as for previous samples. The result is that the PMMA 495, which requires lower dosage levels to achieve the same size of structure when compared to PMMA 950 forms a cavern under the PMMA 950. The PMMA 950 then acts as a mask (see Figure 7.9 c). The material deposited through this mask no longer contacts the PMMA walls, consequently fewer antennas are disrupted by the removal of the PMMA mask during the lift off step.

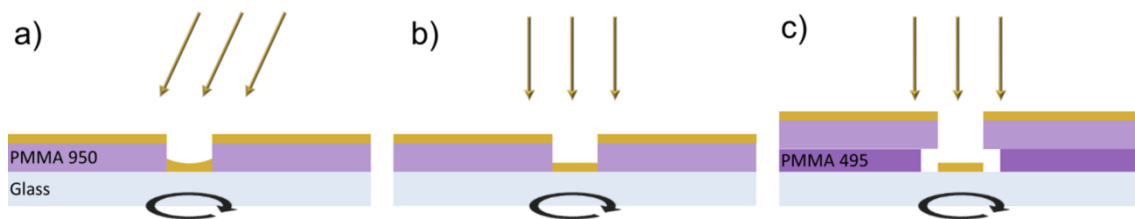


Figure 7.9 – **Effect of evaporation direction relative to the sample and demonstration of the dual layer PMMA technique.** a) schematic showing material build up against PMMA trench walls caused by material evaporation angle. (Samples are rotated during deposition leading to build up on all sides evenly.) b) Example of desired material growth from an evaporation direction perpendicular to surface. c) Schematic showing the results of evaporation onto dual layer PMMA using PMMA 950 and a PMMA 495.

For the samples discussed in this section, Al (Kurt J. Lesker Al 99.999% pure) was deposited at 5 Å/s to a thickness of 25nm followed by a lift off in acetone. The antenna were then exposed to air for an hour to allow them to form its native oxide layer [115, 140] before being stored in vacuum until the samples were characterized and/or subjected to SR mapping.

7.3.2 Characterization

Dark field spectroscopy was performed on the aluminium antennas using the vertical dark field geometry. A linear polarizing filter was used to select for light polarized along the long axis scattering from the antenna. Figure 7.10 a) shows the geometry of Al bar antenna. In this case, when compared to the Au bar antenna, the spacer layer occurs via oxidation of the aluminium surface, so length, width and height not significantly modified by the presence of the oxide layer. Figure 7.10 b) show the DF spectra taken from an Al bar antenna whose size was $L=120\text{nm}$, $W=50\text{nm}$, $H=25\text{nm}$ and $G=30\text{nm}$ with

an expected thickness of Al_2O_3 of $\sim 5\text{nm}$ [140]. The peak resonance is located at 576nm . Figure 7.10 c) shows the simulated resonances of the same antenna geometry in air (solid lines) and water (dashed lines) illustrating the expected red shift when submerged in water. The simulation of the scattering profile in air (black solid line) is a good match to that seen in the DF spectra. There is a small, $\sim 20\text{nm}$, red shift between the DF result and the simulated result. This could be due to a thicker than expected growth of Al_2O_3 or small writing error of the antenna. Figure 7.10 d) shows how the peak scattering response of a simulated Al antenna varies with the thickness of Al_2O_3 layer (grown into the Al antenna). As the thickness increases, the peak scattering wavelength red shifts while the scattering intensity decreases. As we do not have direct control over the thickness of the natural Al_2O_3 layer it and its size effect the resonance of the system it as an extra layer of variability in fabricating specific resonances in our plasmonic structures.

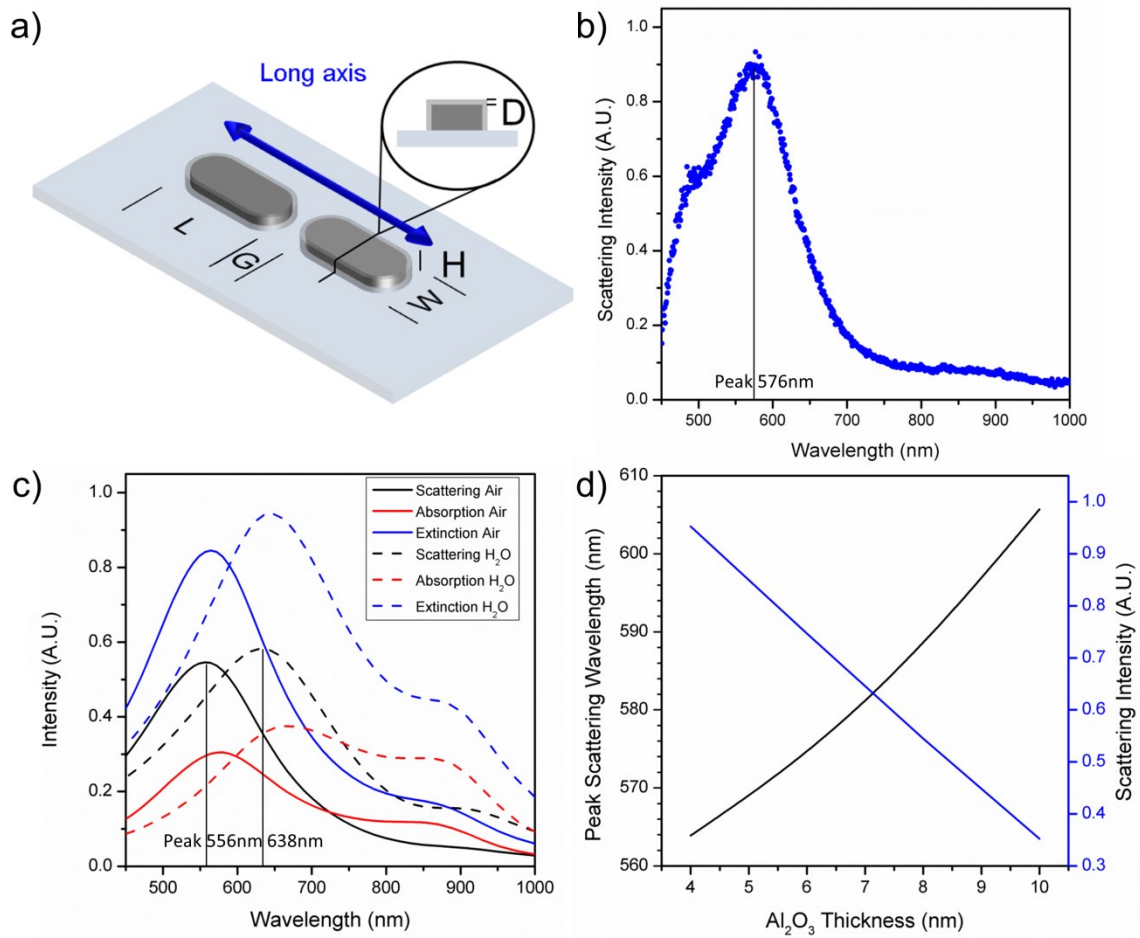


Figure 7.10 - **Dark field and simulated spectra of the optical response of aluminium bar dimers.** a) Schematic of the Al antenna design. Here, D is the thickness of the Al_2O_3 layer that natively forms on the surface. b) DF spectra of an aluminium antenna with (nominal) dimensions of $L=120\text{nm}$, $W=50\text{nm}$, $H=25\text{nm}$, $G=30\text{nm}$ and $D\sim 5\text{nm}$. c) Simulated scattering, absorption and extinction spectra for an Al antenna, on glass, in air (solid lines) and water (dashed lines). d) Scattering peak wavelength (black) and intensity (blue) variation with change in aluminium oxide growth

Having demonstrated that the individual Al bar dimer antennas will have an optical response on resonance with a 640nm excitation source, a design employing two such antennas in close proximity was developed. The purpose of such a design was to demonstrate the clear ability of the SR setup to spatially distinguish two hotspots within a sub-diffraction area. A schematic of this geometry is shown in Figure 7.11 a). The antennas are separated by a sufficient distance, 300nm, to produce little interaction of the antennas, and hence little shift of the resonance peak of the system relative to a single antenna, when illuminated along their long axes, as seen in Figure 7.11 b). The DF spectrum also shows a good agreement with the simulations shown in Figure 7.11 c),

with a slight redshift being present, as was also seen for a single antenna. No SEM were taken for dual dimer antenna as the sample damaged by the SR measurements. Some SEM of single Al dimers were taken early on in the experimental run for this sample that showed good geometry however, the image quality was very poor.

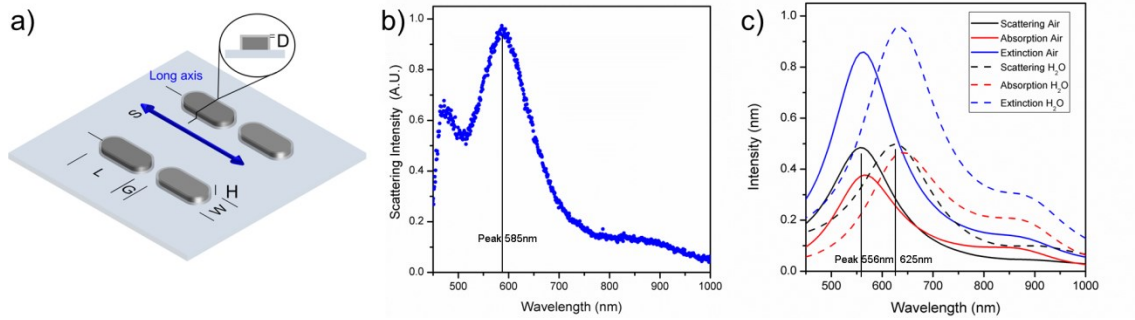


Figure 7.11 - **Dark field and simulated spectra for double aluminium bar dimers.** a) Schematic of the double Al antenna design where S is the separation between the two dimers. b) DF spectra of an aluminium antenna with (nominal) dimensions of L=120nm, W=50nm, H=25nm, G=30nm, S=300nm and D~5nm. c) Simulated scattering, absorption and extinction spectra for the antenna on a glass substrate, in air (solid lines) and water (dashed lines) when illuminated with light polarized along the long axis of the antenna.

A simulation of the gain in the EM field squared ($|E|^2/|E_0|^2$) generated around the aluminium dimer antenna, when illuminated with 640nm light linearly polarized along the long axis of each antenna, is shown in Figure 7.12. Based on the results seen in section 7.2.4 for an Au antenna, two individual circular hotspots should be mapped in the gap regions from these double Al dimer antennas.

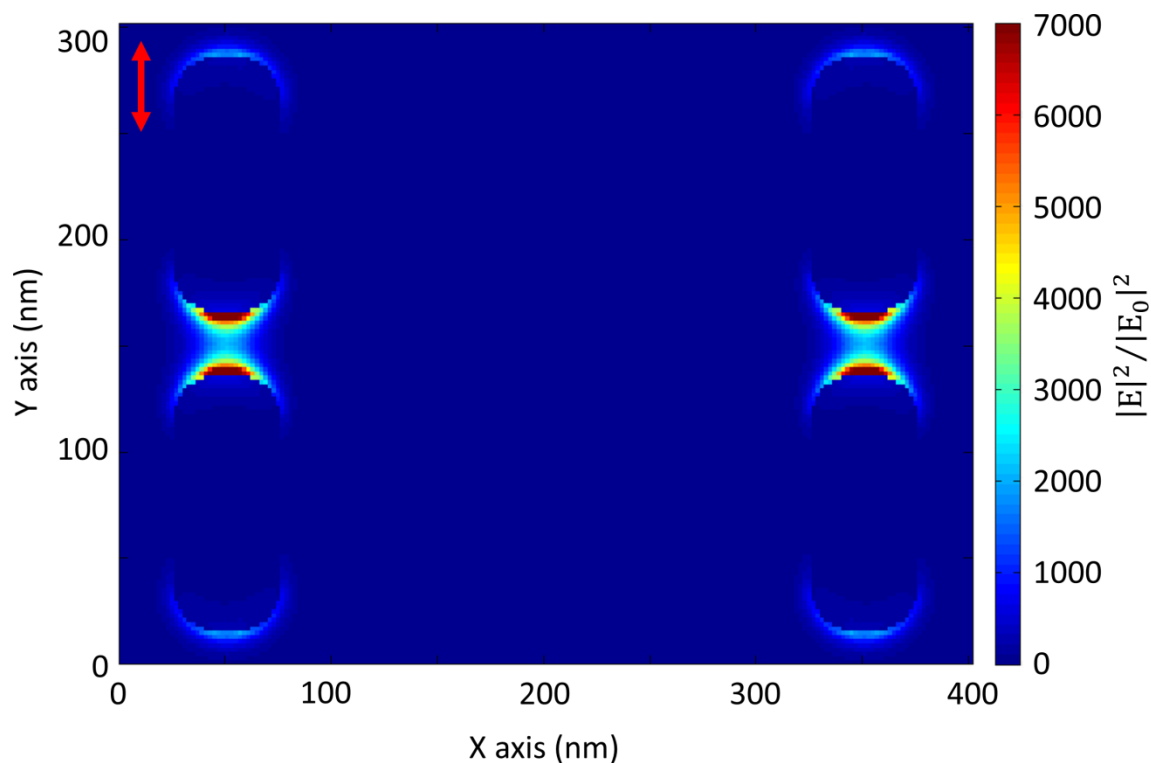


Figure 7.12 - **Simulated $|E|^2/|E_0|^2$ for dual dimer setup.** The polarization orientation of 640nm illumination light is indicated by the red arrow and is aligned along the long axis of each antenna, exciting the antennas across their gaps. The antennas have dimensions of L 120nm, W 50nm, H 25nm and G 30nm and are separated by 300nm. The outer 5nm of the bars are made up of an Al_2O_3 spacer layer and the simulation was done on a glass substrate in a water medium.

7.3.3 Method

The optical setup procedure for the aluminium dual dimer sample was identical to that previously discussed for Au antennas. To improve stability and increase image sequence acquisition time, the stabilization system described in section 6.5 was implemented. The tracking system was calibrated for the defocus of the tracking antenna via a monitored z scan of the piezo electric stage and this defocus gradient was used in combination with PID weights of $k_p = 0.2$, $k_I = 0.5$ and $k_D = 0.001$ to maintain the sample focus. These weights were selected after extensive testing of the tracking system. These values subsequently served as starting values when adjusting the PID settings for new samples.

100 μL of DI water was added to the surface and followed by 20 μL of 10nM of Rhodamine 800. Tracking was enabled after the system was brought into focus and left to settle. The SR system was then illuminated with a 640nm laser for a sequence of 60000, 100ms images, with a down time of 30ms/image, giving a total run time of

~1h50m looking at a set of aluminium dual dimer antennas with dimensions $L=120\text{nm}$, $W=50\text{nm}$, $G=0\text{nm}$ and $S=300\text{nm}$. Following this, the data was processed and localized to produce localization field plots of the antenna.

7.3.4 Results

Figure 7.13 a) shows the x -projection of localized data points for a dual dimer antenna. Some spurious and unexpected localization points (circled in black) were observed in the distribution. Some data points are disconnected from the lower intensity data at the same x position. This implies that this data is spurious. For any peak value at a single xy position we would expect many other lower values to be collected from that same position leading to a smooth climb to a peak value. These 'jumps' in intensity with no linking data points cannot be a product of any expected intensity variations at a single position due to early molecule desorption or orientation. If we also examine the variance vs photon count Figure 7.13 b), a set of data points are disconnected from the main data set (again, circled in black). The spurious points in the variance plot b) correspond to the unexpected localization data in a). These points are attributable to the simultaneous adsorption of molecules in each gap of both antennas. The fluorescent light collected is then made up of two overlapping PSFs. When localized, this combined PSF returns a position between the two real positions with an intensity that is the sum of the two individual overlapped PSFs. Due to the malformed nature of the combined PSFs it gives the localization a high variance for the given intensity. Removing these data points we achieve a much better quality of the envelope, Figure 7.13, that contains the data about the fluorescent enhancement field results at each of the individual antennas. Addressing the interaction rate of the experiment we see that in 60000 frames we identify 12848 PSFs. This gives an approximate probability of 21% that an individual frame contains a fluorescent PSF. Assuming that each hotspot has an equal likelihood of being interacted with the probability of two overlapping interactions is $\sim 4\%$. This gives an estimate of the number of spurious double points of 2600. In when we perform the variance filtering the number of points removed is 2713 which seems to fit quite well with our estimations.

In Figure 7.13 d) two enhancement peaks can be seen, corresponding to the gaps of the individual antennas and showing the expected spacing of almost exactly 300nm, a clear demonstration of the power of the SR technique to resolve multiple closely spaced hotspots.

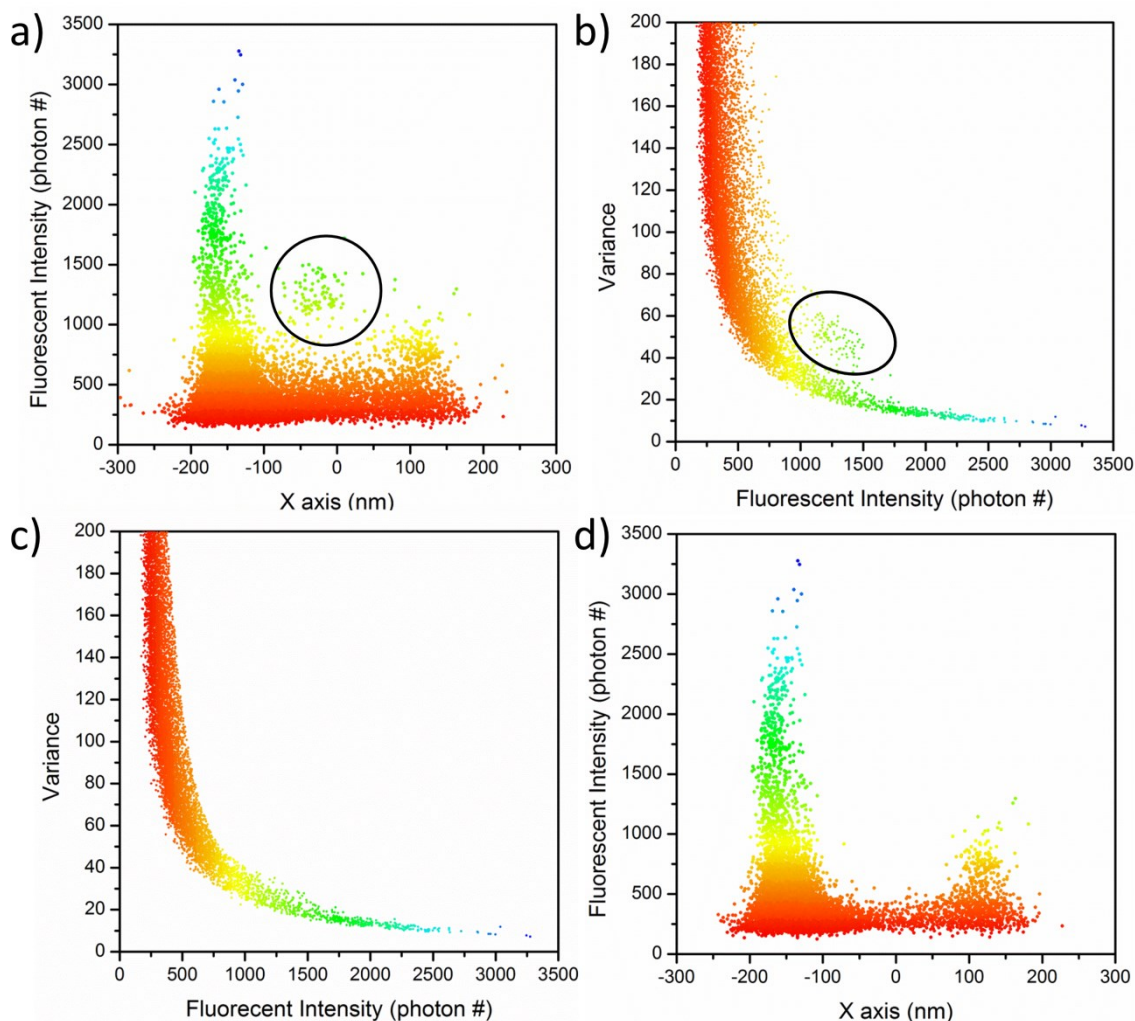


Figure 7.13 - **Localization results from a set of dual Al dimer antennas separated by 300nm.** Samples were illuminated in TIR with a 640nm laser, with light linearly polarized parallel to the long axis of the antennas to generate hotspots in the gaps. a) An x -axis projection of the localized data showing unexpected spurious points (circled in black). b) The variance of the data plotted in a) with intensity with spurious points again circled in black. c) Variance intensity data after filtering out data outside of an allowed variance range. d) Localization data projected onto the x axis after variance filtering.

Following the variance filtering, localization data was processed into skin average images. Examples of these skin averages are shown in Figure 7.14, overlaid on representative antennas. Pixels in these images correspond to 8x8nm bins were the

average of the top 3 values are used to map out the variation in the localization distribution envelope. These envelopes form two separated by 300nm – corresponding to the position of each antennas gap. The fluorescent gain values in these gap areas typically range from 12-22 times the background rate, as shown for a couple of representative antennas in Figure 7.14 b) & c). However, similar to the case of the Au dimers, there are some exceptional antennas that display much higher than average gain values. An example of this is shown in Figure 7.14 d). This variations is likely due to small structural variations that are beyond our current fabrication techniques to control and these results demonstrate how susceptible the performance of plasmonic devices can be to nm level variations.

An unexpected part of the localization distributions is the low light level localization present between the active antennas. The localizations stop abruptly in the spaces beyond the left and right extremities of the antenna pairs and are not present anywhere else on the sample beyond the active antenna regions. This will be addressed later in the text.

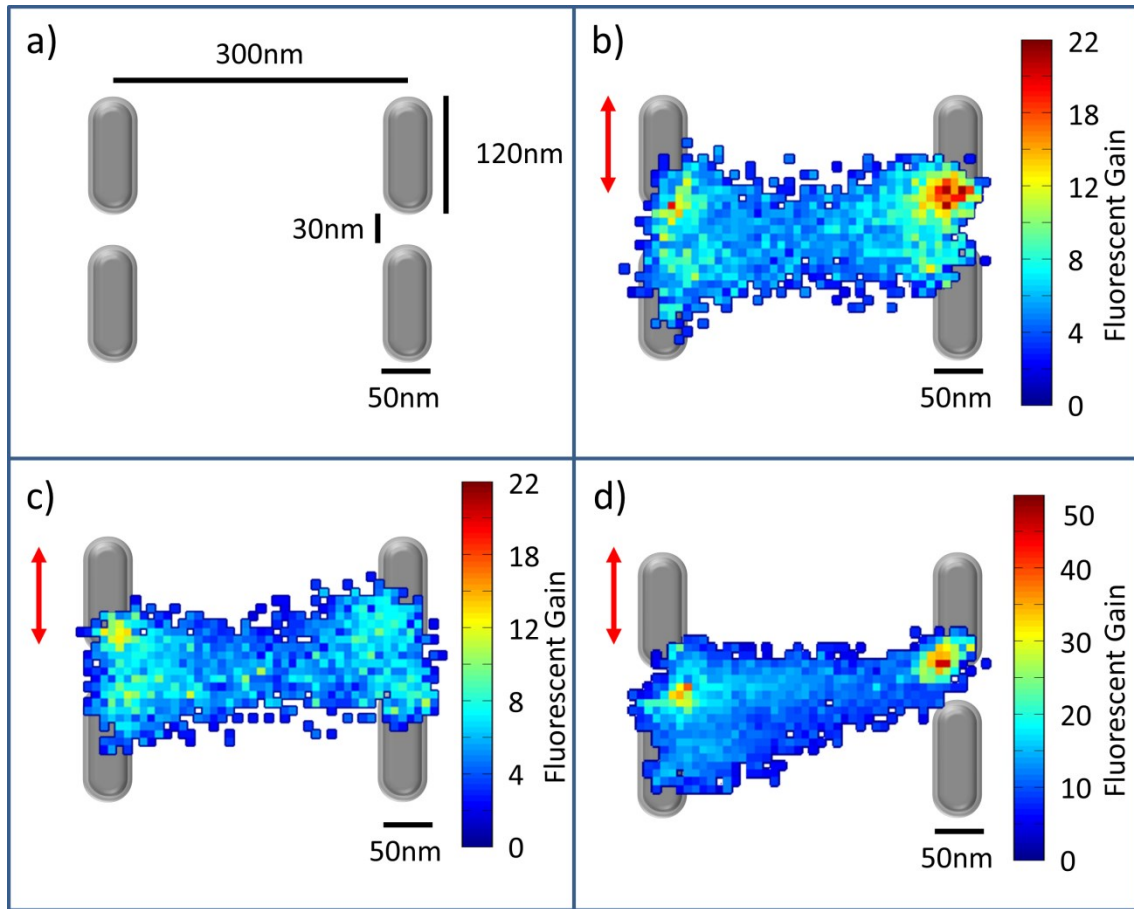


Figure 7.14 – **The localization skin average field result for various dual Al bar dimers.** Samples were illuminated in TIR with linearly polarized light orientated along the long axes of the antennas as indicated by the red arrows. a) A schematic of the antenna geometry. b) & c) Representative localization field examples of common field distributions generated by dimer pairs. d) Localization field distribution for notably enhanced right lobe.

7.4 Probing the Near-Field of an Aluminium Dolmen

One of the initial goals of this research was to study polarization switchable EM field distributions of a single antenna. This is motivated by the strong dependence of the internal field distribution on the polarization of the illumination source for many plasmonic structures. When exciting along the short axis of a dimer antenna, little fluorescence enhancement was seen under the experimental conditions used here – as such, the conditions were unsuited to mapping changes in near-field hotspots with changes in the incident polarization. Therefore, more exotic structures that have multiple possible gap orientations for excitation were required. Such structures have unique field distributions that arise with different polarizations of the illuminating light.

To this end, the geometry selected was that of a dolmen. Dolmen structures have been studied in depth in [141]. A key feature of the dolmen structure is that orthogonal illuminating polarizations can produce enhancements in either one or two gaps, leading to two distinct scenarios.

7.4.1 Sample Fabrication

Al dolmen samples were fabricated using the same procedure documented in 7.3.1. Al was deposited to a thickness of 25nm before lift-off. The thickness of the native oxide layer is again assumed to be 5nm [140].

7.4.2 Characterization

The geometry of the dolmen is shown in Figure 7.15 a). The dimensions of the antenna were designed to be $L=160\text{nm}$, $W_1=110\text{nm}$, $L_2=150\text{nm}$, $W_2=110\text{nm}$, $H=25\text{nm}$ and $G=30\text{nm}$. The gap was the same between all bars and the two lower bars are symmetrical. The DF spectra were taken for long and short axis illumination (directions indicated by the blue and red arrows in Figure 7.15 a)). The DF spectra are shown in Figure 7.15 b) with the curve colours matching their respective polarization orientation colours. Simulations were performed for a dolmen on a glass substrate for light polarized along the long and short axes, as shown in Figure 7.15 c) and d), respectively. Solid lines corresponded to simulations performed in air while dashed lines correspond to simulations performed in water. The refractive index data comes from Palik. Comparing the DF spectra with the simulated long axis scattering response in air (Black solid line in part c)) we see a good general agreement in peak position between measurement and simulation with both display twin peaks at $\sim 600\text{nm}$ and $\sim 900\text{nm}$. The change in relative intensity of the DF spectra peaks with respect to the simulation is likely due to the effect differences in illumination and collection geometries [127] This agreement is, however, not seen for the short axis case. Simulations of the short axis scattering, Figure 7.15 d), show a large, $\sim 100\text{nm}$, blue shift when compared to long axis scattering. DF results show only a small blue shift, $\sim 1/3$ the size of the simulated shift. It is possible that this discrepancy arises due to the optics used to acquire the spectra, as their efficiency is poor towards the short end of the spectrum. The magnitude of disagreement seen here is greater than would be expected, however, even when

considering this issue. A similar shift in the peak position was also observed for DF results taken from different dolmen.

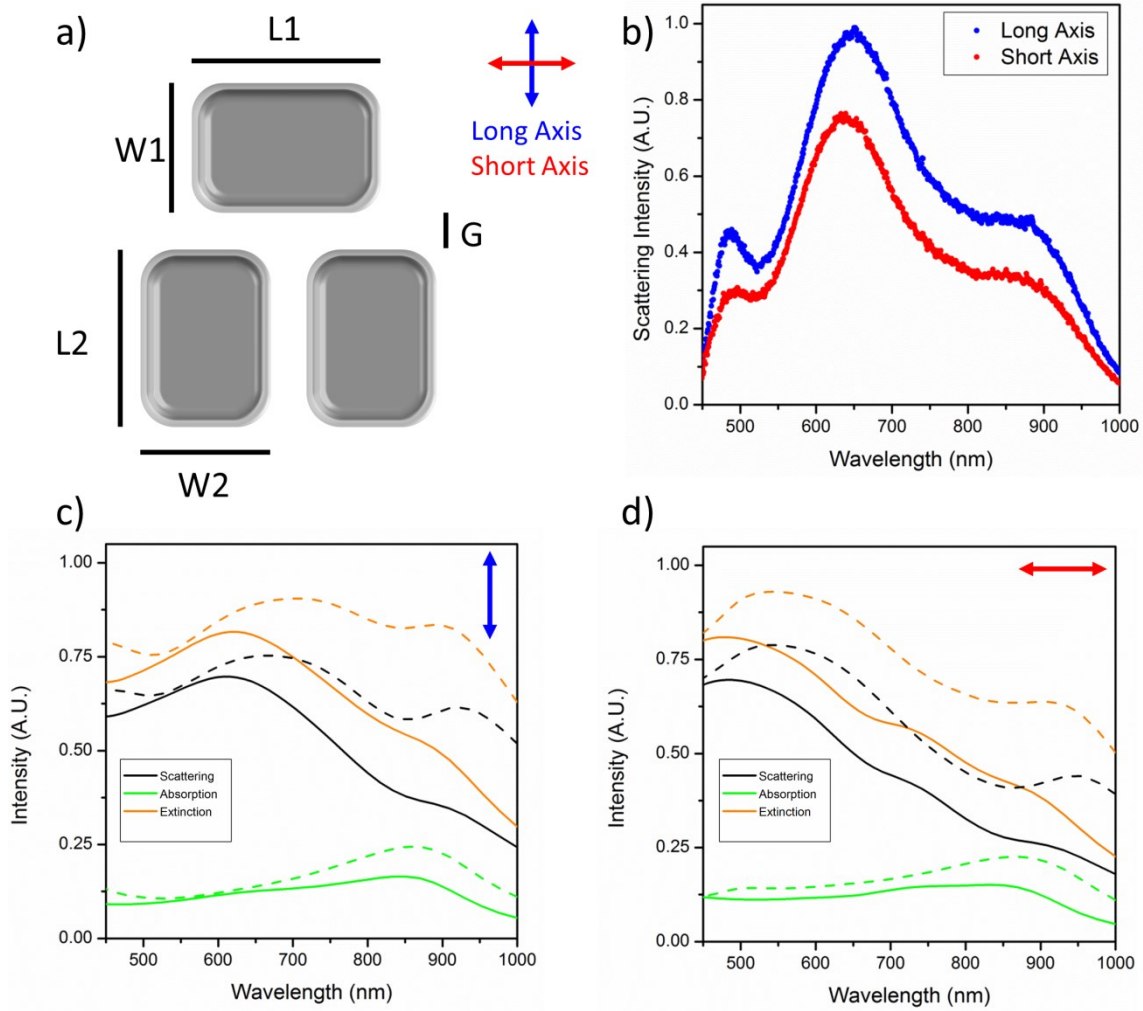


Figure 7.15 – **Dark field and simulated spectra of an Al dolmen.** The dimensions of the dolmen were L1 160nm, W1 110nm, L2 150nm, W2 110nm, G 30nm, and H 25nm. a) a schematic of the Al dolmen structure where the long and short axis directions are indicated by the blue and red arrows, respectively. b) Dark field spectra taken using the vertical dark field geometry using a linearly polarizing filter to select resonances polarized along the long and short axes (blue and red points, respectively). c) Simulated spectra of scattering, absorption and extinction spectra (black, green and orange lines respectively) for long axis illuminated dolmen made of aluminium on glass with a 5nm Al_2O_3 coating. D) Simulated spectra of the short axis resonance for the dolmen. Simulations were done in both air and water (solid and dashed lines, respectively).

Simulation of the near-field EM squared gain ($|E|^2/|E_0|^2$) distributions for long and short axis polarized illumination are shown in Figure 7.16 a) and b), respectively. These simulations display the desired switchable field distributions for the dolmen. Specifically, the structure shows a clear switch from two separated hotspots for long axis

polarized illumination to a single strong hotspot for short axis polarized illumination, a result that would be expected to be repeated in the SR imaging process.

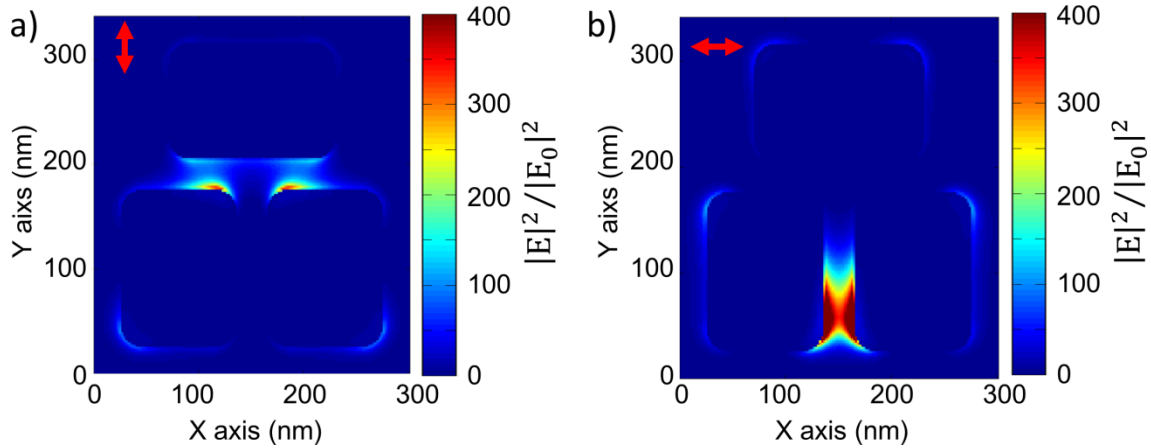


Figure 7.16 – **Simulation of EM $|E|^2/|E_0|^2$ near-field distribution around Al dolmen antenna.** Antenna were illuminated with 640nm light polarized in the direction indicated by the red arrows. a) Long axis polarization illumination. b) Short axis polarization illumination.

7.4.3 Method

The sample was mounted onto the microscope and 100 μ L of DI water was added to the surface. The sample was then brought into focus and TIR illuminated with a 30mW 640nm laser. The system was then left to settle and refocused. 20 μ L of 10nM Rhodamine 800 was added to the surface. Tracking was then engaged on the surface using PID weighting parameters stated in 7.3.3 Method. An image run of 20000 images was then taken with an 100ms exposure time and 30ms/image down time giving a sequence time of ~43minutes. The image run was taken for long and short axis polarization and the data was then processed and localized followed by variance filtering to remove spurious points. These localization fields are then overlaid on schematics of tri-disk antenna imaged. As no direct relation can be made from fluorescent position to schematic the overlays are done qualitatively. We cannot exactly know xy position however the orientation of the localization field is known.

7.4.4 Results and Discussion

The data was then processed into skin average distributions. The results were overlaid on top of a schematic of the dolmen structure, drawn to scale, and are shown in Figure

7.17. The results were not as expected from simulation. Instead of the EM hotspots being localized to the gaps, the localization fields showed peak enhancements over the bars themselves. The orientation of the samples was verified several times. Figure 7.17 a) and b) are examples of a dolmen illuminated with light polarized along their long axes. Two separate hotspots are seen, along with some stray localization points. The origin of these stray points is deemed to be the third (top) bar, and the alignment of the hotspots over the schematic was determined by best overlapping these points. When illuminated with a polarization along the short axis of the dolmen, Figure 7.17 c) and d), the localization distribution demonstrates a switching behaviour, as was desired, however the localizations are seen over the top bar. Moreover, the twin localization patches over the lower two bars are seen to remain active. This unexpected behaviour arises due to the failing of the free space approximation used widely in localization microscopy – a subject considered in more depth in the following chapter before we return to the SR mapping of our antennas.

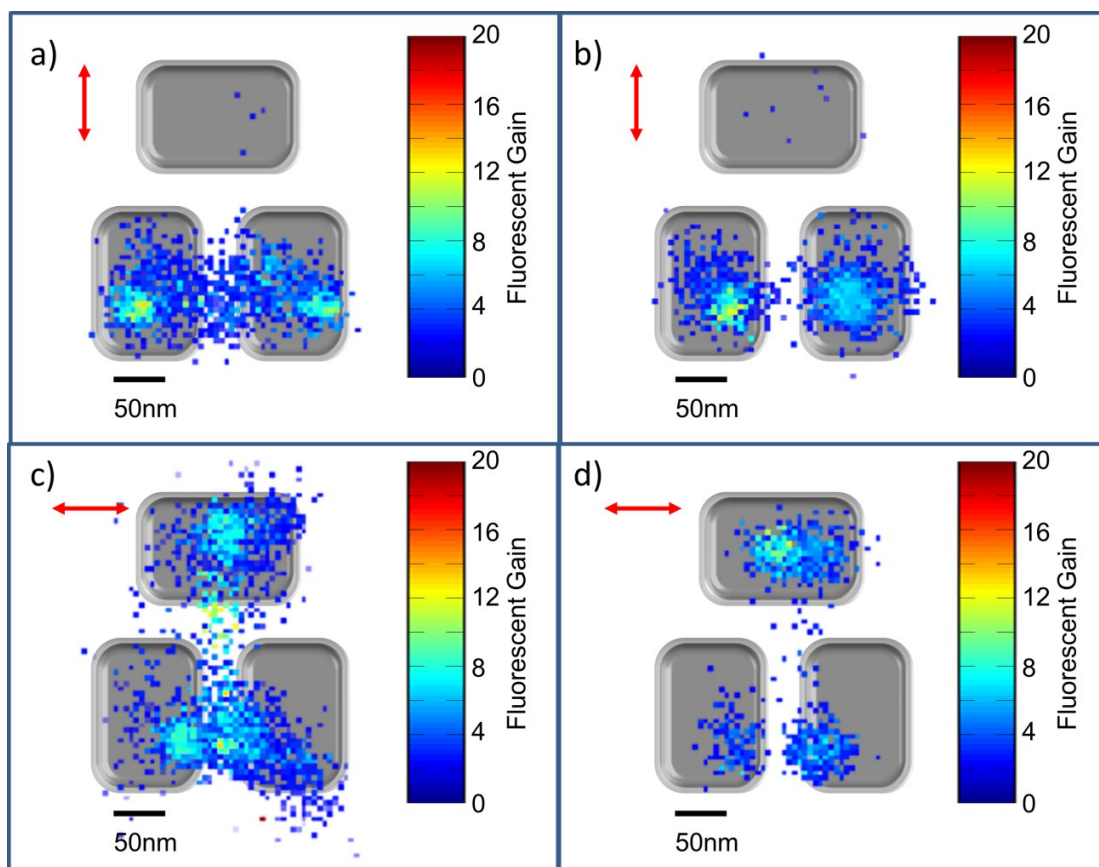


Figure 7.17 – **Skin average localization field distribution results for Al dolmen.** Samples were illuminated in TIR with linearly polarized light whose polarization direction is as indicated by the red arrows. a) & b) Localization distributions for long axis polarized illumination. c) & d) Localization distributions for short axis polarized illumination.

8. Breakdown of the Free Space Approximation

Localization microscopy was initially developed with the imaging of biological samples in mind. These samples can be selected such that they are largely optically homogeneous, therefore, emitted fluorescence from molecular markers is fairly unimpeded before collection and subsequent localization. Because of this the localization, via a Gaussian fit, is a fast and reliable method for determining the position of this approximately free space emitting molecule.

Problems arise when a molecule's emission is strongly affected by interaction with the surrounding environment. This can lead to the distortion of the PSF and a resultant shift between the real position of the molecule and the apparent position of the molecule achieved through Gaussian fitting localization.

In attempting to probe plasmonic near-fields using fluorescent molecules there is a significant interaction between the emitted light and the plasmonic system, leading to a dramatic distortion of localization distributions for such systems.

Initial papers in the field of SR imaging [137, 142] did not discuss or address this issue, however, as the field progressed, the problem became more apparent and its significance more appreciated.

An early example of this is shown in Figure 8.1 [22]. The Willets group, which produced these results, has been one of the leading groups in the field of molecular localization to probe molecule/plasmon interactions. Here, fluorescently labelled double-stranded DNA is bound to the surface of a gold nanorod, covering the surface. The strands keep the fluorescent molecules a few nm from the surface of the gold. The majority of the molecules were forced into a temporary dark state, preventing them from emitting and ensuring that light from only a single active molecule is collected at any one time. This collected light is then localized and the active molecule is allowed to change. By processing many molecules, the expectation was to build a localization field that had a very similar size of the Au rod. TAMRA fluorescent molecules were used as probes and Figure 8.1 a) shows the frequency histogram built up from the fluorescent data. The

histogram bins are 4.34nm in size and make up an event density plot. Figure 8.1 b) shows the fluorescence intensity distribution obtained for the antenna. The event frequency distribution from part a) is then overlaid upon an AFM image of the antenna and shown in Figure 8.1 c). It can be seen that the localization distribution is significantly smaller than the true size of the antenna even though molecules are covering the entire surface of the antenna.

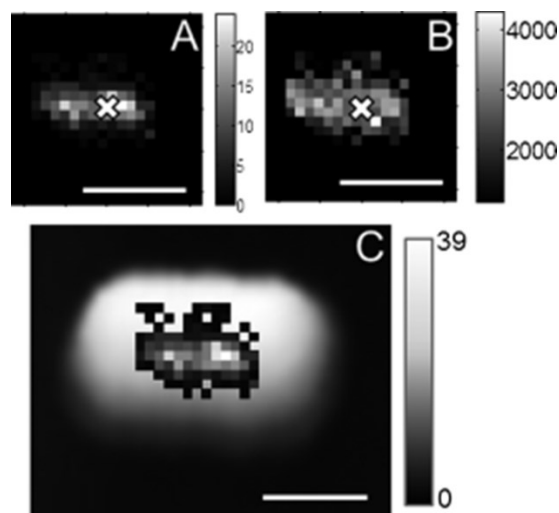


Figure 8.1 – **Fluorescent single molecule triplet state mediated SR imaging of AU bar antenna** using TAMRA fluoresce dye bound to the surface using ligands. A) Frequency histogram. B) Spatial intensity map. C) AFM image of gold rod overlaid with frequency histogram from part A). (Height scale bar in nm and bin pixels 4.34nm in size) Reproduced with permission from. [22].

One of the models that may explain this “shrinkage” was explained by considering that of an emitting dipole generating a dipole image in the nearby structure. This image produces secondary photons that are emitted from the structure itself. These secondary photons undergo a Young’s type interference with the directly emitted photons from the dipole, causing a significant distortion in the imaged PSF [23]. Figure 8.2 a) and b) show an analysis of this type of interference produced by a dipole interacting with a nearby silver nanowire reproduced with permission from [23]. Figure 8.2 part a) is schematic diagram illustrating the interaction. The back dipole induces currents in the nanowire producing secondary radiation. Both secondary radiation and direct radiation are collected by the camera. Light from these two sources interferes, producing distorted PSFs, as shown in Figure 8.2 b). Here the PSFs for a dipole whose polarization is aligned perpendicularly to the nanowire direction (red) and aligned parallel (blue), are

shown. The polarization orientations relative to the sample are shown in the insert to Figure 8.2 a). The distortion in the PSF can be easily seen and the localized centres are marked with small open circles which in both cases are positioned away from the true position of the dipole (marked in solid black).

These distorting effects depend on the amount of light transmitted to the far-field direct from the dipole and antenna respectively.

A second power transfer explanation was made by considering an optical coupling between a dimer nearby a NP. Photons that couple into the plasmonic modes of the NP are reradiated to the far-field directly from the NPs position. This leads to a PSF generated directly over the antenna that when mixed with the PSF generated by light directly emitted to the far-field from the molecule leads to a localization position shifted towards the position of the antenna. The power transfer pathways available are shown in Figure 8.2 c), which is reproduced with permission from a 2015 paper by J. S. Biteen's group reference [21]. In the Figure 8.2, P_1 and P_4 are the powers transmitted to the far field from the dipole and the antenna, respectively.

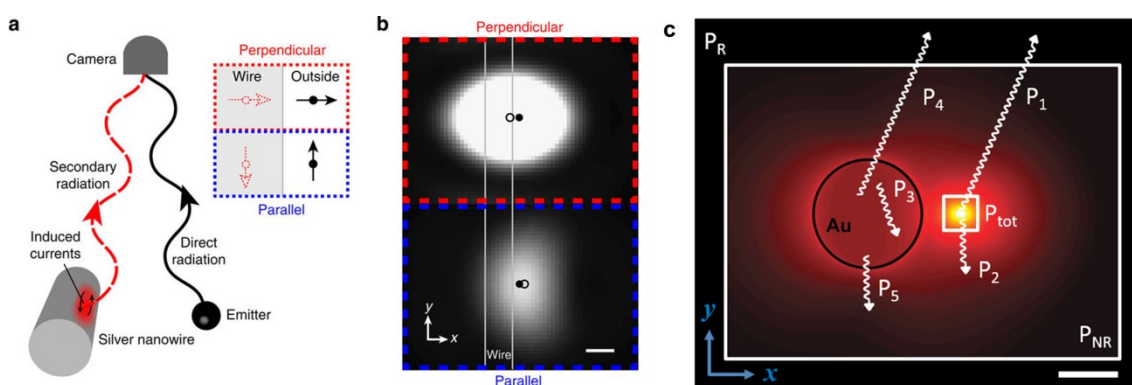


Figure 8.2 – Image dipole interference and antenna dipole power transfer. a) An illustration of the interference between light emitted directly from a dipole and secondary light produced from an induced dipole image in nearby a silver nanowire. b) calculation of far-field diffraction spots for dipoles perpendicular (red) and parallel (blue) to wire for a dipole 30nm from wire's surface. Parts a) and b) reproduced with permissions from reference [23] Nature Communications 2014. c) Power dissipation path ways for a dipole interacting with an Au disk. P_1 Power radiated directly to far-field from dipole. P_2 Power lost through non-radiative energy transfer to environment from dipole. P_3 Power coupled to Au disk then dissipated into the Au due to material losses. P_4 Power couple to Au disk then radiated to the far-field. P_5 Power coupled to Au disk then lost via non-radiative energy transfer to surrounding medium. Reproduced with permission from [21] Nano Letter 2015

One of the most convincing experimental demonstrations of the mis-localization effect was by Wertz *et al.* [21]. In this work, the interaction between Cy5.5 fluorescent molecules and Au disks illuminated with a 640nm laser using a PAINT setup (similar to the one documented in this manuscript) was studied. The disk antennas were tuned to be on resonant with the illumination wavelength which was polarized circularly to produce a near-field profile of EM field squared gain shown in Figure 8.3 a). The circular polarization combined with the shape of the antenna produces a ring of field enhancement that we would expect to be reflected in the localization distribution. We would expect then, if localized positions correspond to true molecule positions, that a localization distribution would show this ring structure with strong enhancement at the edge of the disks, corresponding to the EM enhancement shown in Figure 8.3 a). However, due to the power coupling into the antenna from the dipole, shown in Figure 8.3 b), the position for the molecules were mis-localized, forming a sharp peak above the centre of the disks as shown in the intensity enhancement plot in Figure 8.3 c). The 2D insert shows how the localizations overlay the 90nm disk Au disk being probed.

This is extremely convincing evidence of mis-localization as the gold antennas are uncovered by any spacer layer. This means that any molecules that are directly bound to the surface of the gold would have their fluorescence totally quenched [50]. In comparison with other works where the dipoles are held at a distance through the use of ligands or dielectric spacer layers, in the case this quenching of emission at the surface of the Au itself means that any localizations appearing to come from molecules over the disk, must have been due to mis-localization of molecules whose true position is off the disk. The paper also discussed how the coupling distances observed were much larger than initially expected, an effect not discussed in detail here.

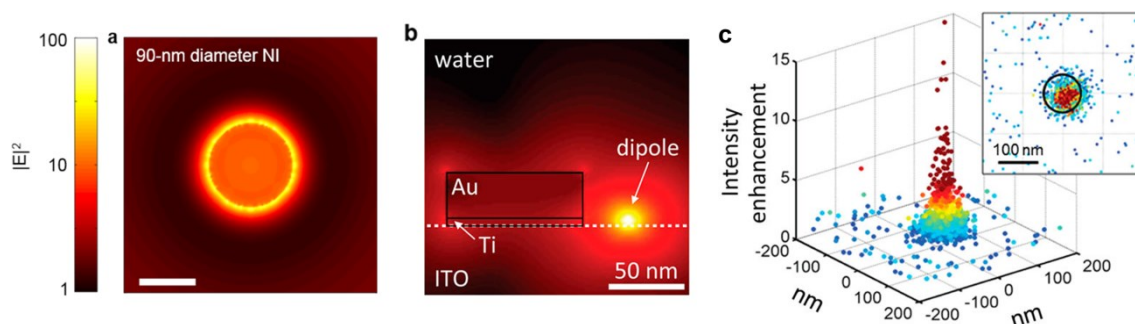


Figure 8.3 – **Mis-localization Cy5.5 dye interacting with an Au disk.** a) FDTD simulation of enhanced field around 90nm diameter gold nanodisk excited at 640nm with circularly polarised light. b) Dipole interaction with an Au nanodisk with a separation of 30nm. c) Localization result for Cy5.5 interacting with a 90nm Au disk. Reproduced with permission from [21] Nano Letter 2015.

Although the possibility of using models and advanced fitting techniques to retrieve the true position the molecules was discussed and considered for this work, the added complexity of modelling greatly restricts the aim SR images to act as direct probes of the plasmonic environment, as results must be interpreted against simulations generated for known structures. The inherent variability in fabrication and the presence of defects in the structures then lead extensive struggles and difficulties in interpreting the results.

To overcome the mis-localization problem, the spectral decoupling of fluorescent emission from the plasmonic antenna being imaged was considered for this work. To continue with the study of polarization dependent EM near-fields a new dolmen based antenna was designed to be resonant at $\sim 405\text{nm}$. Using dyes that emit away from this resonance (and hence will not interact with the antenna during their emission) decouples the emission from the antenna. FDTD Simulations of the near-field distribution for dipoles emitting at various wavelengths next to this 405nm resonant antenna were performed. As the emission wavelength was shifted off resonance from the antenna, the amount of field coupled into the system from the dipole is reduced, as shown in Figure 8.4. As such, the mis-localization effect should be reduced, enabling the SR method to act as a direct probe of the EM field enhancement around the structure and eliminating the need for complex simulation and interpretation.

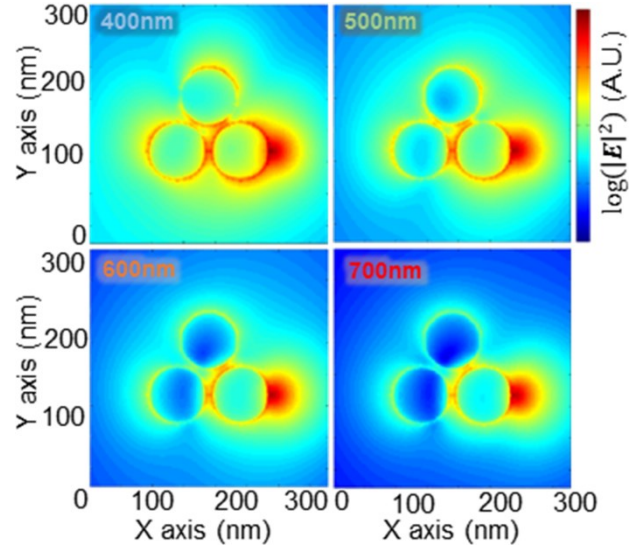


Figure 8.4 - FDTD simulations of a dipole placed 10 nm to the side of a plasmonic Al tri-disk antenna emitting at different wavelengths. Emission from the dipole is tuned from an on resonance condition (400 nm) to progressively more off resonance with respect to the LDOS peak of the system.

To confirm our hypothesis that the fields can be decoupled in this manner, FDTD simulations were performed (see Figure 8.5). Light from a dipole was projected into the far-field, with dipole antenna interactions being accounted for. These far-field projections can then be localized in the same manner as an actual measured PSF to find the apparent position of the dipole. In doing this, it was possible to study the effect of the interaction with a plasmonic structure on the dipole's apparent position. The simulation technique is discussed in more detail in section 5.3 Similar simulations have been used in [21, 143].

In Figure 8.5 an Al disk antenna with a diameter of 80nm and a height of 25nm (see insert) is simulated interacting with a dipole emitting at 400nm (black points) at a distance d from the edge of the disk. The light from the simulation is projected into the far field onto a flat plane at which point the resultant diffraction limited PSF is localized. This gives an “apparent” position of the dimer. The position of the dimer is then changed to be progressively further from the disk edge in 5nm steps. By plotting the apparent position against the true position (dashed lines) of the dipole with respect to the edge of the antenna it is possible to see how the apparent position of the dimer is effected by the emitted light coupling to the disk.

It can be seen that for dipoles close to the edge of the disk, their apparent positions appear to be near the centre of the disk. As the dipole moves away from the disk, the shift toward the centre is significant until $d \sim 40\text{nm}$. At this distance, a reverse effect is seen, with the apparent position curve crossing the true position dashed line and the dipole appearing up to 20nm further away from the disk than it actually is. This positive shift in the dipole position is also mentioned in [23]. As the dipole is moved even further out, the localized position of its fluorescence converges with its real position (indicated by the black dashed line).

The simulation was then repeated for an emission wavelength of 550nm (red points). It can be seen that for light emission shifted away from the resonance frequency of the antenna, there is a significant reduction in the difference between apparent position and actual position. Dipoles close to the edge have their mis-localization reduced by 60% . This is due to the decoupling of the emission of the dipole from the disk.

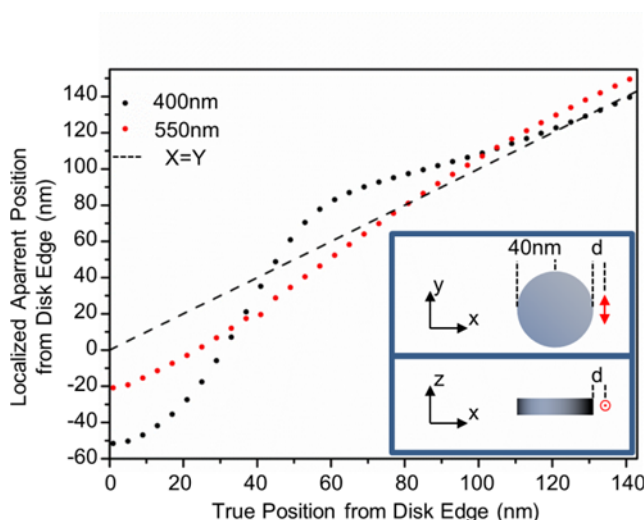


Figure 8.5 - **Effect of coupling between a dipole and an antenna on the apparent localized position of the dipole.** Results are shown for a dipole emitting on resonance at 400 nm (black dots) compared to one emitting off-resonance at 550 nm (red dots) near an Al disk. The dashed line corresponds to the perfect localization at the true position of the dipole.

As discussed in section 6.4, fluorescent molecules typically have Stokes shifts of $< 50\text{nm}$ between peak absorption and peak emission wavelengths. Because of the relatively broadband response of plasmonic antennas, it is very hard to decouple the emission of these dyes such that one can have strong excitation enhancement of the molecules

(absorption resonance with the antenna) without having the emission couple to the antenna and displace the localization position. By selecting a dye molecule with a large Stokes shift, however, it is indeed possible to notably reduce mis-localization to probe, and more accurately map, the near-field of a polarization selective antenna.

9. Probing the Near-Field of Aluminium Plasmonic Structures Using Stokes Shifted Dye Molecules.

In order to decouple the dye emission from the plasmonic resonance, it is necessary to separate the plasmonic resonance from the spectral region of the emission to the largest degree possible. To accomplish this, the plasmonic antennas were redesigned to be resonant at $\sim 400\text{nm}$. This required the use of smaller antennas. As we intended to continue the study of structures with polarization dependent near-fields that are excited across changing antenna gaps, we opted to miniaturise and simplify the dolmen geometry to a set of three equally sized Al disks arranged in an equilateral triangle formation.

In water Al antennas suffer degradation through oxidation. This degradation was found to be greatly enhanced due to antenna heating [144]. A protective self-assembled polymer layer solution has been explored in the literature [145], however, the polymer itself fluoresces over our observed spectral range. Dimethyl sulfoxide (DMSO) provides an extremely stable medium for the aluminium antenna. No degradation was observed over the course of the experiment. This was verified by pre and post DF spectroscopy that the antennas remained unaltered over the course of our measurements.

9.1 Sample Fabrication

Due to the need for smaller structure size and with the implementation of perpendicular evaporation deposition, as discussed in section 7.3.1, the dual layer PMMA technique was abandoned. The smaller size of the structures made it harder to achieve good quality shape using dual layer PMMA. A single layer of PMMA 950 was used to achieve excellent lift off quality with extremely low antenna loss rates. The PMMA layer was deposited via spin coating and was written and developed as documented in section 6.2. To improve upon previous Al film qualities a much higher deposition rate of $20\text{\AA}/\text{s}$ was used and deposited at a base pressure of $\approx 2 \times 10^{-7}\text{Torr}$. This was done to reduce the aluminium oxide contamination within the aluminium layer [146] and to increase adhesion to the glass [147]. Deposition was followed by an acetone lift off step.

9.2 Characterization

SEM images were taken of the antennas following the SR experiments. Due to aluminium's low atomic weight it gives extremely poor contrast with the substrate due to their similar atomic masses. This means that Al antennas can be very difficult to image in a scanning electron microscope. To address this, samples were coated in a 3nm layer of platinum. This dramatically improves SEM image quality, but makes antenna appear slightly larger than their actual sizes (with gap sizes appearing smaller). Another drawback to coating samples in Pt for imaging is that once coated the samples are unusable for further SR experiments as the Pt layer cannot be removed. Consequently, SEM images were taken after all other experimental work with the antennas was completed.

Examples of the fabrication quality are shown in Figure 9.1. The top row images are of antenna arrays featuring a) $L=120\text{nm}$ $H=30\text{nm}$ Al rods, b) $D=70\text{nm}$, $G=30\text{nm}$ and $H=30\text{nm}$ tri-disks and c) $D=70\text{nm}$ $G=30\text{nm}$ quad-disks. The bottom left antenna in the array shown in Figure 9.1 a) is a tracking marker disk, present in the centre of each array and used to give consistent tracking and position correction for each array during the long measurement time. The arrays are sized such that a single full array fits in the field of view of the EMCCD camera allowing 48 antennas to be observed in a single image run. Each antenna is separated from its neighbour by $2.5\mu\text{m}$.

Individual antennas from each respective arrays are shown immediately below the array in the lower row of Figure 9.1 (parts d)-e)). The quality of antennas for their small scale, is very high, however, it should be noted that there is some variation between each individual antenna shown in the arrays. The variation generally comes in the form of misplaced disks reducing or closing the gaps between antennas or left over PMMA blocking some gaps. This can be seen easily in Figure 9.1 e), where the top left and bottom centre gaps show some bridging material between disks. The presence of this bridging material is reflected in the SR localization fields, where blocked antenna gaps often lead to inactive arms of the antenna and reduced gap sizes lead to higher

fluorescent gains. For this reason, each antenna must be individually analysed for the current work.

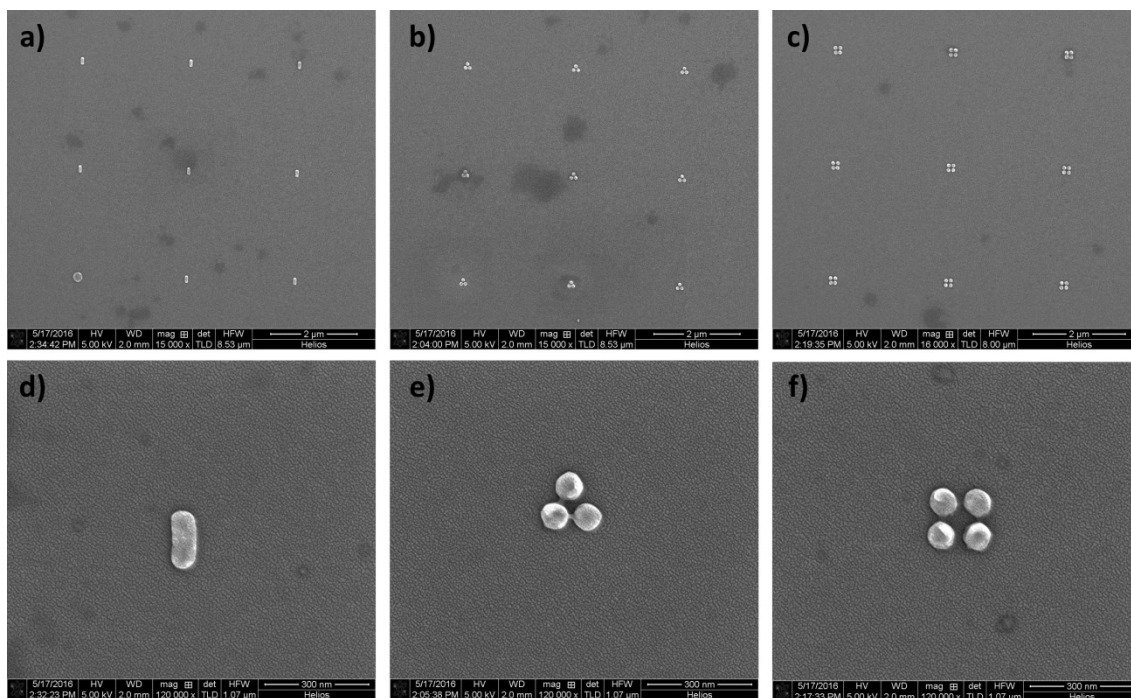


Figure 9.1 – **SEM images of Al plasmonic antenna** fabricated for stokes shifted dye studies. Samples were coated in a 3nm layer of Pt to improve SEM image quality. a) 120nm rod array b) tri-disk antenna array c) quad-disk antenna array d) A single L=120nm W=50nm rod e) a single tri-disk antenna f) a single of quad-disk antenna.

To perform DF spectroscopy on new Al antenna some significant upgrades and modifications to the DF setup documented in section 6.3 were necessary. The typically used fibre, DF objective and camera were not suitable to collect and recover light at 400nm. This combined with the halogen lamp used as the light source, which does not extend far into the blue end of the spectrum, meant that the DF setup as initially constructed did not clearly show the resonant behaviour of the antennas in the violet-blue spectral region.

A UV objective was used to improve the initial collection. This objective (M-Plan APO NUV 50x, NA=0.42) requires the use of the side illumination geometry shown in Figure 6.8 a). A Nikon Intensilight C-HGFI mercury lamp, which provides better illumination in the blue and UV, was used in place of the conventional halogen lamp. Finally, a

FG600AEA Thorlabs fibre was used to couple light from the microscope to a new UV window coated Princeton Instrument CCD camera, improving collection efficiencies.

Spectra were then taken of Al disk to be compared with FDTD simulation of the scattering spectra. Figure 9.2 a) shows a schematic of the Al disk design which are coated in a layer of Al_2O_3 . The DF results in Figure 9.2 b) are for 80nm, 100nm and 120nm (black, red and blue lines respectively). Even with the new components taking DF of these antenna was challenging leading to the lower quality of DF results. However the antennas still display the expected red shift with increase radius. FDTD simulations in Lumerical were then performed for these disks and shown in Figure 9.2 c). There is a reasonable agreement between DF and simulation which suffers only a 50nm red shift from simulation to DF. Due to the difficult nature of the dark field measurement of these blue antenna, simulation were to select and plan antenna resonances.

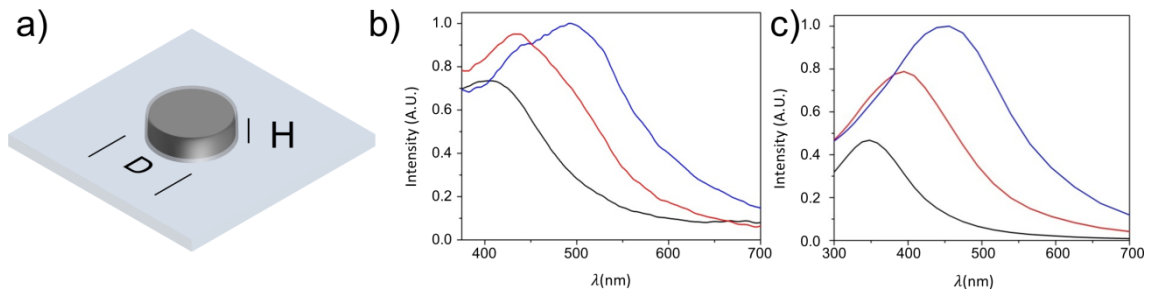


Figure 9.2 - **Scattering from Al disks.** a) schematic of single Al disk coated in a $\sim 5\text{nm}$ layer of Al_2O_3 with diameter D and height H . b) side illuminated dark-field spectra for 80, 100 and 120nm diameter Al disks with heights of 30nm (Black, red and blue lines, respectively). c) FDTD simulated scattering spectra for Al disks of 80, 100 and 120nm diameter with a 30nm height whose outer 5nm layer consisted of Al_2O_3 performed using Lumerical. The 5 nm oxide accounts for the surface oxidation of the Al that inherently occurs.

In order to study polarization dependent near-fields and switching, a tri-disk antenna design was selected. This produces similar near-field switching to that of the dolmen antenna, however, is more easily fabricated consistently at smaller scales. Figure 9.3 a) shows a schematic of these tri-disk antennas. The construction is made up of three equally sized disks of diameter D and height H arranged in an equilateral triangle such that the gap G between each disk and its neighbours is the same. An SEM image of a platinum coated tri-disk where $D = 70\text{nm}$ and $G = 30\text{nm}$ is shown in Figure 9.3 b). The

measured size of this antenna, when accounting for the Pt layer, is almost exactly as desired.

An FDTD simulation of the scattering response of this antenna geometry in DMSO is shown in Figure 9.3 c) with the peak absorption wavelengths for pacific blue and pacific orange dyes marked with blue and orange dashed lines, respectively.

The antenna scattering resonance (black solid line) is centred at ~ 400 nm and, as is typical for Al antennas on a glass substrate, is quite broad [148]. The maximum absorption peak wavelengths of both PB and PO are both centred near 410 nm. Both lines can be seen to be strongly on-resonance with the Al tri-disks.

To study the coupling and enhancement of emitted fluorescent light in close proximity to such a plasmonic antenna, FDTD simulation of the total decay rate enhancement γ/γ_0 were made using dipoles. The method is discussed in section 5.3. Briefly, the simulations were done using two separate methods, one directly monitored the power of the dipole and the power transferred to the far-field and then calculated a decay rate enhancement through these direct observations. The second made a calculation of the Dyadic Green's function and from this calculated a decay rate enhancement factor. These results were used to check and confirm each other.

As shown in equation (6.8) the total decay rate enhancement is made up of the radiative decay rate enhancement γ_r/γ_0 and non-radiative decay rate enhancement γ_{nr}/γ_0 . This total decay rate enhancement expected for a dipole located in the centre of the bottom gap of the tri disk antenna is plotted in Figure 9.3 d). The peak emission wavelengths for pacific blue and pacific orange are indicated to show the expected recombination enhancements for these dyes (blue and orange solid lines, respectively).

The peak emission wavelengths in DMSO for these dyes are ~ 450 (PB) and ~ 575 nm (PO). The emission of PO, therefore, shows a large Stokes shift compared to PB, as both dyes have similar peak excitation wavelengths. Analysis of Figure 9.3 d) shows that the emission of PB is further within a strong total decay rate enhancement band of the system. As such, one expects that the emission from PB will couple more strongly to the

plasmon modes of the tri-disk system, causing a mis-localization of the emitting molecules position as discussed in the previous chapter. In the coupled case, the emitter takes on the emission profile of the simple metal antenna [149].

It is worth noting the peak in enhancement occurs at $\sim 900\text{nm}$. This peak is due to a large non-radiative contribution due to the interband transition for Al. This causes a large increase in non-radiative channels for $\lambda > 650\text{nm}$.

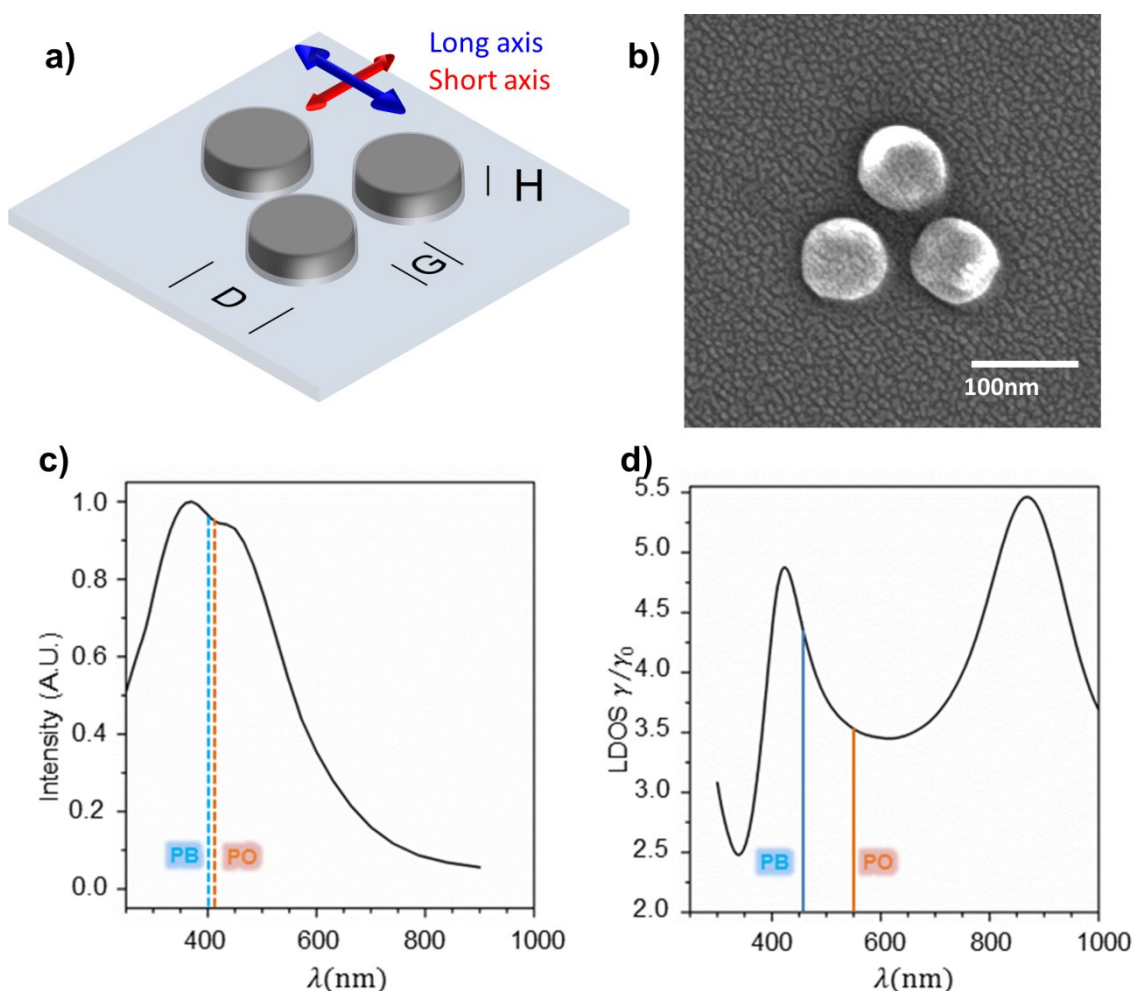


Figure 9.3 - Spectral characteristics of the tri-disks antennas. a) schematic of tri-disk coated in a 5nm layer of Al_2O_3 . Disks are all diameter D , height H , and gap size G . and with the long and short axis direction marked with blue and red arrows respectively. b) SEM of Al tri-disk with $D=70\text{nm}$ $G=30\text{nm}$ and $H=30\text{nm}$. To improve SEM quality, samples were coated in a 3nm layer of Pt. c) Simulated scattering spectra of an Al tri-disk system in DMSO (black) overlaid with the maximum absorption wavelengths for PB and PO dyes (blue and orange dashed lines respectively). d) γ/γ_0 total decay rate enhancement of the system overlaid with the maximum emission wavelengths for PB and PO dyes (blue and orange lines, respectively).

9.3 Method

After characterization the sample was mounted onto the microscope. 100 μ L of DMSO was added to the surface followed by 20 μ L of 10nM dye DMSO solution. The sample was then brought into focus on an array of D=70nm G=30nm tri-disk. The sample was illuminated through a laser line filter (Z405rdc Chroma) using a 405nm nm laser diode source (Coherent Cube) .The laser light was filtered out of the image using dichroic (Z405rdc Chroma) and emission filters (ET420LP Chroma). Tracking was then engaged on the surface using PID weighting parameters stated in section 7.3.3. Image sequences of 60000 frames were acquired, where frames had a 100ms exposure time with $\sim$ 30ms of dead time between acquisitions, giving a sequence length of 130 minutes. Sequences were taken for long and short axis polarizations of the tri-disks using both pacific blue and pacific orange dye. A separate sample was use for each dye to prevent cross contamination of the different dyes. The two samples were made simultaneously in a single fabrication run to try and mitigate fabrication differences. Data was then processed and localized forming data sets of positions and fluorescence intensities. This data was then filtered for spurious results from failed localizations. Specifically, the results were filtered for position, variance and intensity. To retrieve the information for the distribution surface, the results were binned into 10x10nm bins and an average of the top three brightest localizations within that position was taken. This formed the skin average distribution.

9.4 Results and Discussion

After data analysis of both dyes, for both polarizations, the results were overlaid over diagrams of the expected tri-disk geometry. Figure 9.4 a) shows the super resolution localization map obtained for the PB dye coupled to our tri-disk structure, where it is resonant with both absorption and emission, and illuminated with a 405nm laser. The polarization of the illumination source is indicated by the red arrow and each “pixel” in the image corresponds to an area of 10nm x 10nm. Figure 9.4 c) shows FDTD simulations of the near-field electric-field distribution for both polarizations. It should be noted here that the slight asymmetry in the y-polarized near-field electric distribution is due to the TIR illumination geometry used in these simulations. In the case of x-polarized light, one

can see that an electric field enhancement is produced between the bottom two disks. When these results are compared to the x -polarization results in Figure 9.4 a), one readily sees that this gap enhancement is reflected in the intensity increase in this gap region for the tri-disk nanoantenna. When the illumination is switched to y -polarized light, the total fluorescence intensity for PB decreases but the localization map remains very similar in its overall spatial distribution compared to the x polarized one. The antenna-emitter coupling is independent of illumination polarization as it depends on the orientation of the molecule, which we have no control over. This is because although the illumination field excites molecules whose dipole orientation is aligned with the field they are able to rotate on the surface before emitting. In this way the emission orientation of the molecule is random and the summed light unpolarised. However, the EM field strongly depends on the polarization of the source. Obtaining very similar spatial distributions in the localization maps for both polarizations confirm that the free-space approximation is not valid for an emission coupled dye - the invariance of the localization maps with respect to the polarization is caused by the re-radiation of the emission to the far field via the antenna. If the localization maps were reporting the EM field distribution, we would expect a stronger spatial dependence on polarization.

Figure 9.4 d) shows a simulation of the effect of the available LDOS on the total decay rate enhancement, γ/γ_0 , for a tri-disk nanoantenna for $\lambda = 450, 575$, and 700 nm. This simulation was done via calculation of the dyadic Green's function, $\mathbf{G}$, for the electric field at a position $\mathbf{r}$ due to an x -polarized point source at $\mathbf{r}_0$ using equation (5.4). From the Green's function we can calculate a nanoantenna's effect on the density of states available to the dipole, which in turn allows us to calculate the decay rate enhancement (further details in section Dipole Interaction with Plasmonic Antenna Simulations 5.3).

At 450 nm, the emission wavelength of PB, a large portion of the emitted light gets radiated to the far-field via the nanoantenna. As such, the localization for the coupled case of PB and our tri-discs cannot be relied upon to provide the true location of the molecule. Rather, the localizations reflected in our super-resolution maps are a complex combination of the light radiated from the molecule in a hotspot and light radiated via

the antenna. Disentangling these two effects is not easily accomplished. Attempts to return the true molecular position, via simulation, for a simple plasmonic system have been recently proposed [21, 150, 151]. An examination of the total decay rate enhancement plot at 575 nm indicates a reduced effect on the antenna-coupling phenomena at that wavelength (Figure 9.4 c)). As such, one expects that the localization of PO, which emits at this wavelength, will primarily reflect the EM hotspot's contribution to fluorescence enhancement. By simply decoupling the emission we should be able to minimize unnecessary complexity and interpretive issues with the results.

With these points in mind, Figure 9.4 b) shows the localization maps for x and y polarized light obtained for PO on the tri-disk sample. Notice that the total collected fluorescence scale, F_{TPO} , is $\approx 1/3$ that of F_{TPB} ; a detail we will revisit later in this section. Also to be noted is the x - y broadening of the PO localization map. This is due to the localization position beginning to converge towards the true molecule position and away from the “photonic centre of mass” of the antenna system. This agrees with the results of Wertz *et al.* [21] , as molecules located ~ 90 nm from the antenna are able to re-radiate via the plasmonic system when coupling with the plasmonic states; thus leading to a mis-localization of the emitters' position from the actual position of the antennas, as observed for PB but not for PO. In these PO maps, it is also readily apparent that the localization results show distinct features when the illuminating polarization is rotated – reflecting the polarization dependence of the EM field enhancement observed in the simulation results (Figure 9.4 c)), with a strong correlation to the gap enhancements expected under these polarization conditions. This shows that as the molecule's fluorescent emission moves away from the plasmonic resonance, the localization field map becomes more sensitive to the polarization change of the illumination field. This reinforces the fact that once the number of available plasmonic states to couple into has been reduced, the localization maps reflect better the EM field distribution. Interestingly, the total gain values for both antennas are seen to be comparable, even though PB has both its absorption and emission in resonance with the antennas while PO has only its emission in resonance. In order to understand this result,

we must look more detail at the contributions of the radiative and non-radiative LDOS and how they affect the behaviour of the dyes and the overall tri-disk system.

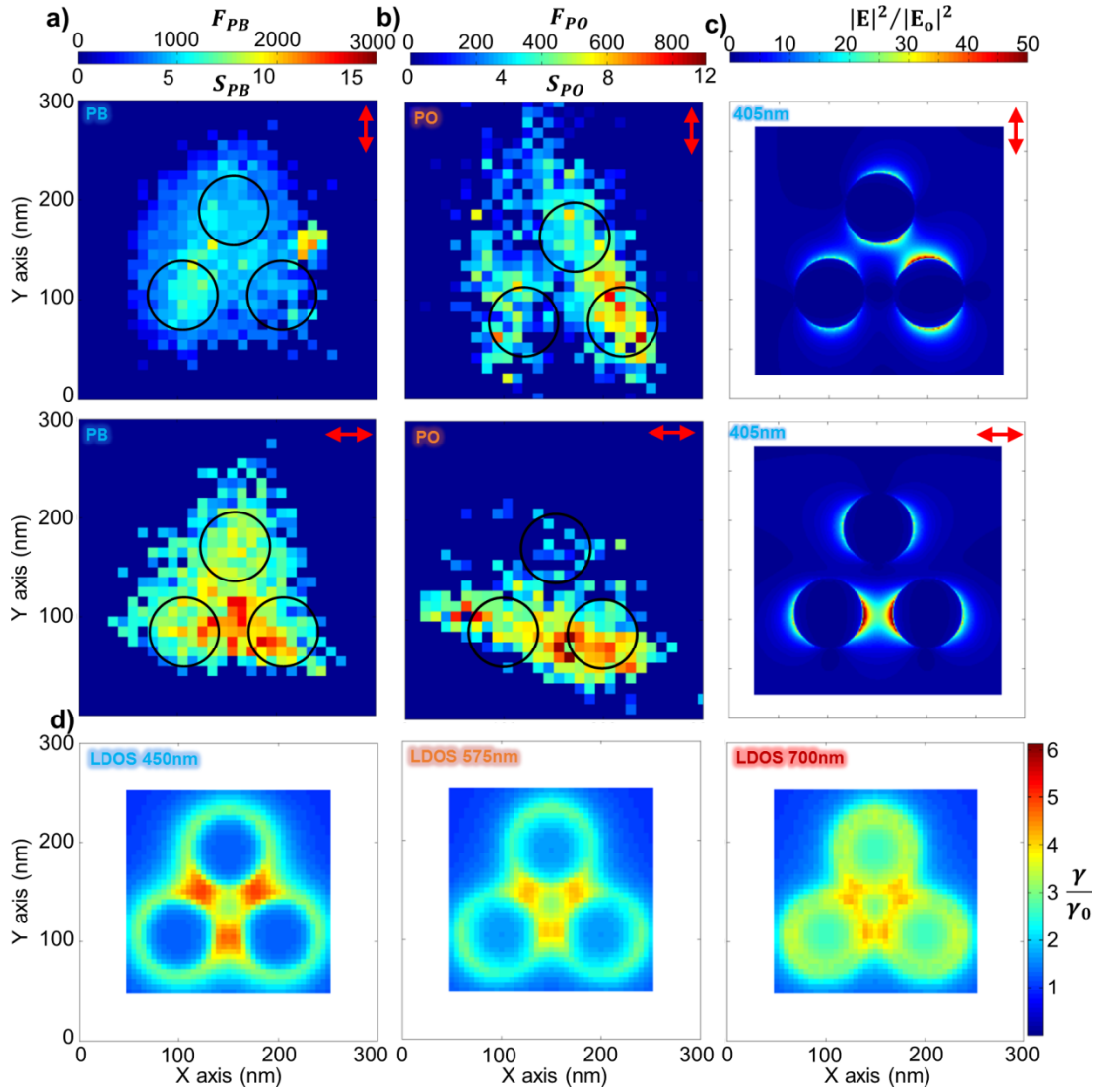


Figure 9.4 - Super-resolution localization maps, EM field enhancements and total decay rate enhancement (due to increase in LDOS) results for tri-disk system. Experimental results for the localization fields for Al tri-disks structures illuminated by a 405nm laser in TIR with the polarization indicated by the red arrow. The colour corresponds to the fluorescent enhancement level S and total collected fluorescence F_T for a molecular probe at that apparent position for the cases of a) Pacific Blue dye and b) Pacific Orange. c) FDTD simulation of the $|E|^2/|E_0|^2$ near-field distribution for TIR illuminated Al tri-disk structures on a glass substrate in water d) FDTD simulations of the effect of the increased LDOS on the decay rate enhancement of a dipole at each pixel position at wavelengths 450, 575 and 700nm.

After analysing the effects of the LDOS and the EM field on the localizations events for both dyes, let us now compute their respective contributions to the total fluorescence

gain values for each dye. We will use this analysis to demonstrate that the localization of PO reasonably represents the EM field around our structures.

As we have mentioned, when the emission wavelength of the dye overlaps with the peak in the LDOS due to the plasmonic structure, leading to an enhanced radiative emission rate, there is a shift of the emission position towards the photonic centre-of-mass of the system that leads to a shift of the localization position. The extent of the mis-localization was illustrated in Figure 8.5 which demonstrated the apparent localized position for a molecule as a function of its position from the edge of a single Al disk. The dashed line showed the case of perfect localization. For a dye molecule emitting at 400 nm (black dotted line), where there is a strong coupling associated with the available plasmonic states – reflected in the increased LDOS at that wavelength – the mis-localization was strong. At 550 nm (red dotted line), however, the emission was decoupled from radiating via the plasmonic structure, as can be seen in Figure 9.5, and the apparent position of the molecule approached its real position (Figure 8.5).

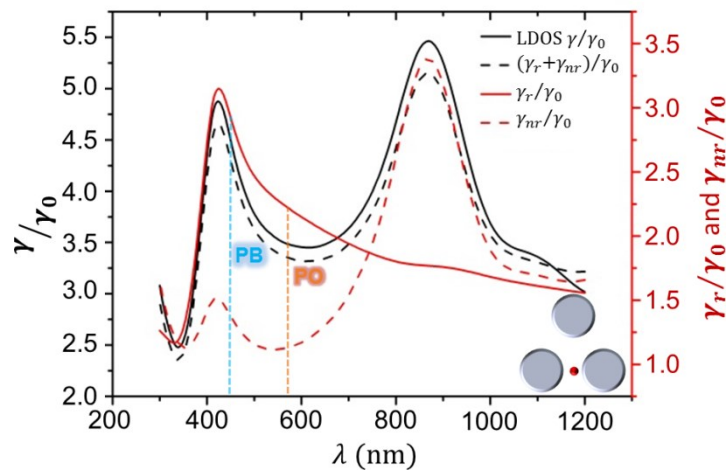


Figure 9.5 - **Spectral response of decay rate enhancement factors.** The total decay rate enhancement (black dashed line), radiative decay enhancement (red solid line) and non-radiative decay rate enhancement (red dashed line) for a combined x - y polarized dipole located at the position marked in red on the insert. The total decay rate enhancement, via calculation of the dyadic Green's function for the structure, is also shown (black solid line).

Firstly, note that although plasmonic structures present a large increase in the number of states for photons to couple into, these states will have both radiative and non-radiative pathways for the out-coupling of this energy [3, 45]. Figure 9.5 shows the

enhancement factor for the decay rate due to an increase in the LDOS available, as well as for the individual radiative and non-radiative contributions for an Al tri-disk antenna. As was seen in Figure 9.4 d), the radiative enhancement via plasmonic coupling for the Al tri-disk antenna is reduced when moving from 450 to 575 nm. Over this wavelength range, these radiative contributions (solid red line in Figure 9.5) are the dominant factor to the overall decay rate enhancement, with only a small contribution from non-radiative pathways (dashed red line). Moreover, one can see that the decay rate enhancement increases at 700 nm, a result not readily expected based on the plasmonic resonance of the antenna alone (see Figure 9.2 c)). This increase is, however, related to an increase of the non-radiative channels for $\lambda > 650\text{nm}$, which correspond to inter-band transitions in Al. We note that although radiative effects are not completely eliminated at 575 nm (the PO emission wavelength) going above 650 nm in the emission of the dye would lead to significant coupling to non-radiative states.

We explore this and other properties of the LDOS for dipoles in close proximity to a the D 70nm and G 30nm Al tri-disk in Figures 9.6- Figure 9.9. The Figures 9.6- Figure 9.8 display simulation results of spatial maps, broken down into individual polarizations and various wavelengths for the total decay rate enhancement γ/γ_0 , the radiative decay rate enhancement γ_r/γ_0 and the non-radiative decay rate enhancement γ_{nr}/γ_0 respectively.

The simulation technique was discussed in depth in section 5.3 but to quickly review, a dipole is placed at an xy position on a plane 10nm above the top surface of an antenna system. A simulation is then performed calculating its spectral responses for γ/γ_0 via calculation of the Dyadic Green's function. At the same time a separate calculation is made, via direct observation of the dipole emission power, antenna emission power and system emission, to calculated γ_r/γ_0 and γ_{nr}/γ_0 . These separate techniques can then be used as a check for each other by the addition of the γ_r/γ_0 and γ_{nr}/γ_0 to a total decay rate enhancement. This comparison is made in Figure 9.5 by the dashed black line which is the result of the addition of the directly observed and calculated γ_r/γ_0 and γ_{nr}/γ_0 rates to the solid black line which is the result of the Dyadic Green's function

calculation of γ/γ_0 both for the same dipole polarised in x and y and positioned in the centre of the lower tri-disk gap (shown on insert). A good agreement can be seen between the two methods.

Colour maps of the simulation results of the decay rate enhancement (via Dyadic Green's function calculation) are shown in Figure 9.6. The simulation is displayed broken down into x and y polarization for the dipole (shown by the white arrows) in Figure 9.6 a) and b), respectively. In the plots each pixel (5x5nm) corresponds to an individual simulation of the decay rate enhancement for a dipole at that pixel's location and at a specific wavelength (400nm, 500nm 600nm and 700nm) for each column, as indicated at the bottom of the figure.

It can be seen from Figure 9.6 a) and b) that the decay rate enhancement for each dipole polarization is highest within the gaps oriented to with the same direction as the polarization. This enhancement drops in the gaps as the wavelength increases however the decay rate enhancement from over the disks increases as the wavelength increases. This can be seen more easily in Figure 9.6 c) which shows the combined contributions for x and y polarized dipoles. Initially for $\lambda = 400\text{nm}$ there is almost no contribution from the disks but by $\lambda = 700\text{nm}$ there is a strong contribution.

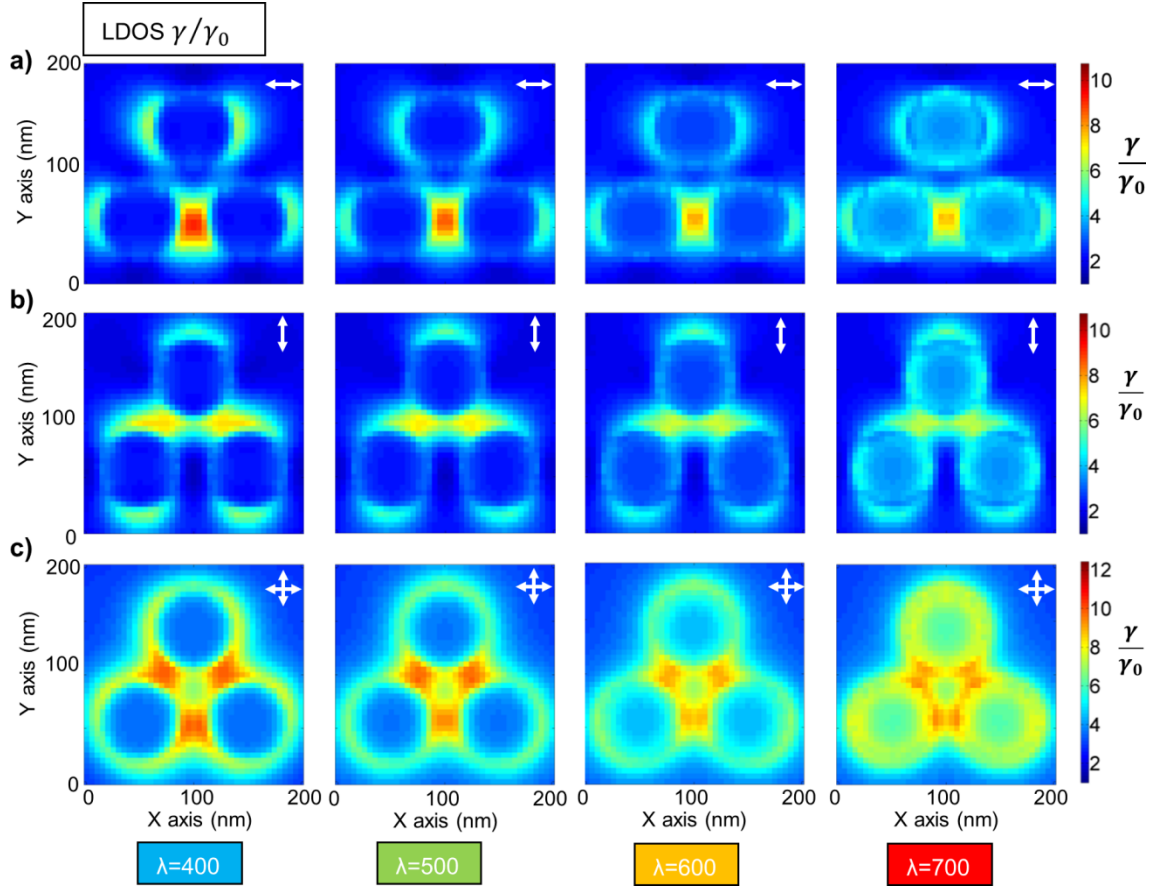


Figure 9.6 - **FDTD simulation of dipole decay rate enhancement due to an increase in LDOS** with tri-disk plasmonic system, scanned over each 5nm pixel location in a plane 10nm above a 70nm radius, 30nm gap, 30nm thickness Al tri-disk structure in DMSO. Plots show the LDOS at wavelengths of 400,500,600 and 700nm. The polarization of the dipole in each plot is given by the white arrow direction. a) The increased LDOS effect on the total decay rate of a dipole polarised in the x direction. b) The increased LDOS effect on the total decay rate of a dipole polarised in the y direction. c) The combination of x and y polarised dipole contributions.

When we compare this with the colour plots of Figure 9.7 which shows the directly observed simulations of the radiative enhancement for all orientations we see a similar diminishment of the enhancement in the gaps. In this case, however, where the decay rate enhancement sees increased enhancements for the locations over the disks at large wavelengths there is no such increase seen when looking at only the radiative enhancement.

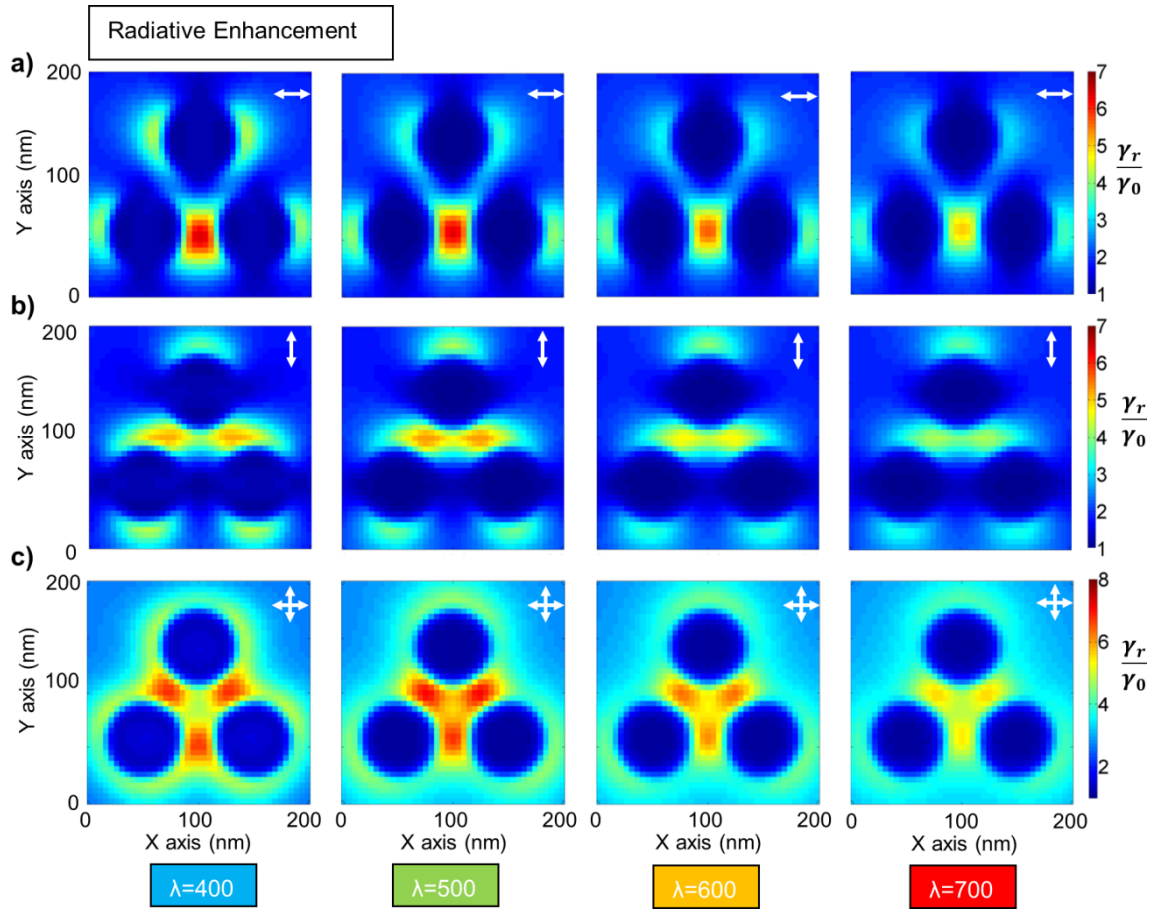


Figure 9.7 - **FDTD simulation of the radiative decay rate enhancement** for a dipole in a tri-disk plasmonic system scanned over each 5nm pixel location in a plane 10nm above a 70nm radius, 30nm gap, 30nm thickness Al tri-disk structure in DMSO. Plots show the enhancement at wavelengths of 400,500,600 and 700nm. The polarization of the dipole in each graph is given by the white arrow direction. a) Radiative enhancement of a dipole polarised in the x direction. b) Radiative enhancement of a dipole polarised in the y direction. c) The combination of x and y polarized dipole contributions.

This increase in the decay rate enhancement shown in Figure 9.6 is due not just to the radiative enhancement in Figure 8.4, but also due to an increase in non-radiative decay rate enhancement as can be seen in Figure 9.8. Part a) and b) show the shape of non-radiative enhancement distribution across the disks for each dipole polarization however the combined polarization shown in Figure 9.8 c) is the most clear. The large increase in non-radiative enhancement located primarily over the disks can easily be seen, showing a stronger effect with increased wavelength. The position of the non-radiative enhancement is very telling of the origin of the effect. As mentioned previously the increase is largely due to the inter-band transition of Al. This means as we approach

the inter-band transition point the energy lost into the Al material increases dramatically, in turn, increasing the non-radiative decay rate enhancement. Because the increase is due to a property of the bulk metal, and not one of the plasmon itself, it is located primarily over the metal and not in the gaps between the disks.

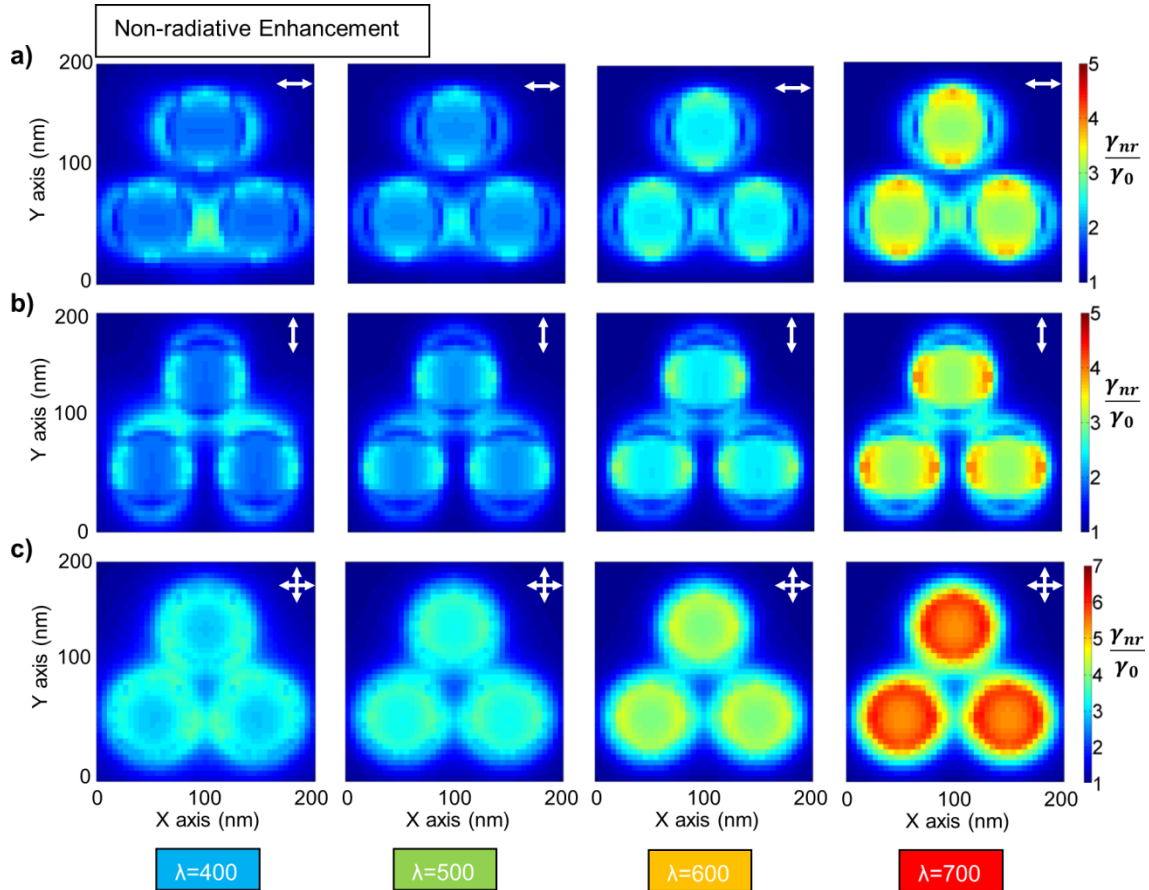


Figure 9.8 - **FDTD simulation of the simulation of the non-radiative decay rate enhancement** of tri-disk plasmonic system scanned over each 5nm pixel location in a plane 10nm above a 70nm radius, 30nm gap, 30nm thickness Al tri-disk structure in DMSO. Plots show wavelengths of 400, 500, 600 and 700nm. The polarization of the dipole in each graph is given by the white arrow direction. a) Non-radiative enhancement of a dipole polarised in the x direction. b) Non-radiative enhancement of a dipole polarised in the y direction. c) The combination of x and y dipole contributions.

This spatial separation of effects is clearly illustrated in Figure 9.9. Figure 9.9 a) shows decay rate enhancement colour plots for a combined xy polarized dipole at 450 and 575nm (Upper and lower plots, respectively). Figure 9.9 b) shows the spectral variation (along the bottom axis) of the emission enhancement for 1D x -slice taken through

centre of the lower gap in the tri-disk structure (the exact position is indicated by the dashed lines in (a)).

It is possible to see how, for short wavelengths and close to the resonance for the plasmonic structure, there is enhancement in the gap immediately. As the wavelength increases large peaks are seen across each disk becoming clearly evident by 800nm. By comparing the radiative and non-radiative contributions in parts c-d) and e-f), respectively, it can be seen that the total decay rate enhancement present at low wavelengths is located in the gap and drops away with increased wavelength and the peaks $\sim 870\text{nm}$ are features of the disks themselves corresponding to non-radiative states.

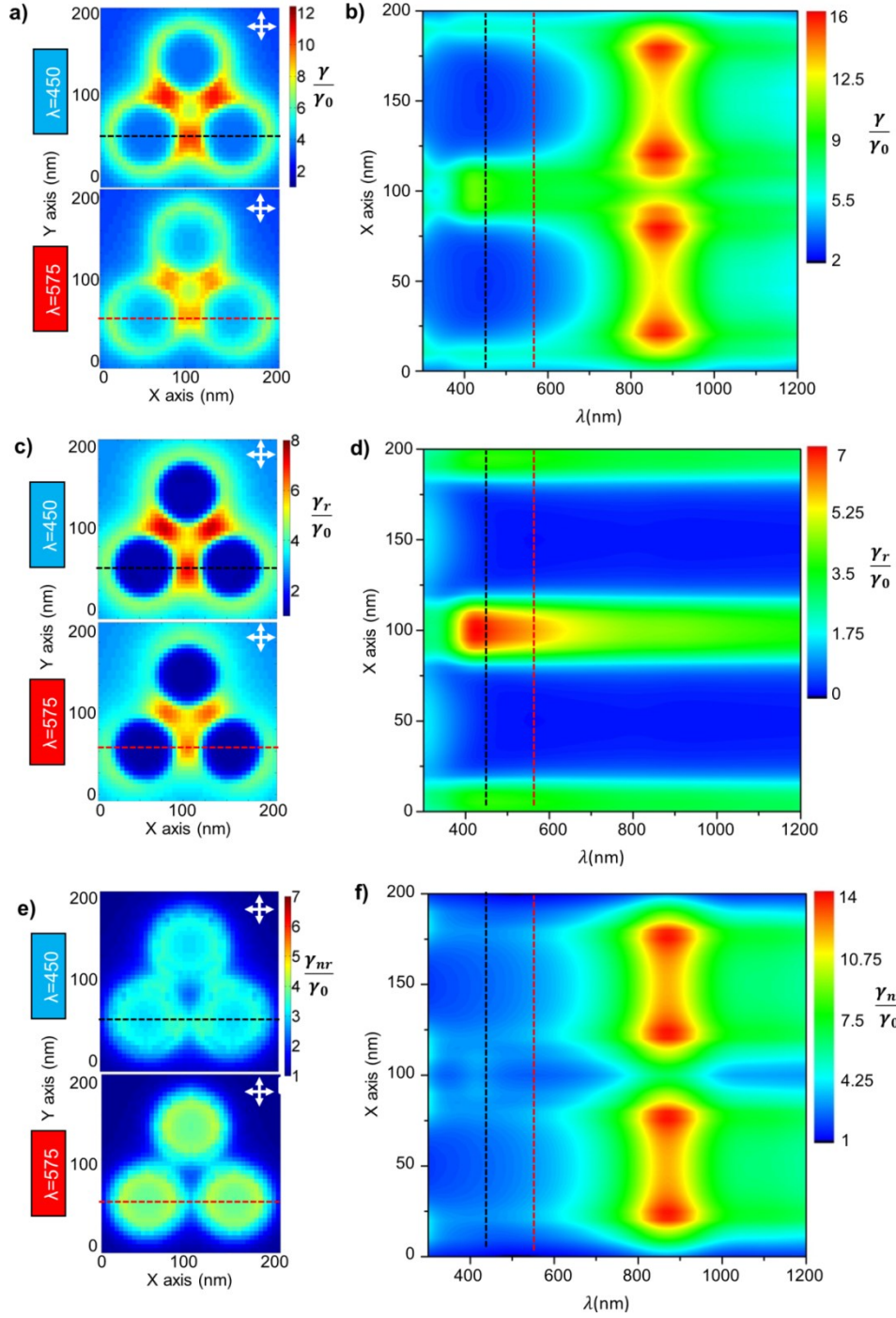


Figure 9.9 - Plots of the total decay rate enhancement due to the increased LDOS, radiative decay rate enhancement and non-radiative decay rate enhancement. The right side column shows the enhancements at the positions indicated by the dashed lines in the left side column in the relevant colour plots for an x - y polarization of the dipole. a) Total decay rate enhancement plot for $\lambda = 450$ nm and $\lambda = 575$ nm. b) Spectral map of total decay rate enhancement along positions marked in a). c) Radiative decay rate enhancement plot for $\lambda = 450$ nm and $\lambda = 575$ nm. d) Spectral map of radiative decay rate enhancement along positions shown in c). e) Non-radiative decay rate enhancement plots for $\lambda = 450$ nm and $\lambda = 575$ nm. f) Spectral map of non-radiative decay enhancement along positions shown in e).

The increased non-radiative enhancement over the disks indicates increased coupling to the plentiful non-radiative states available due to the inter-band transition of Al. This coupling does not affect the localization process, as these states are dark to our observation efforts, however, the brightness of the interactions would be greatly quenched. As such, 575 nm provides close to the best available compromise between these two situations.

Since we are using a predesigned antenna geometry producing a known hotspot we are able to compare the experimental values of fluorescent enhancement in Figure 9.4 a) and b) to the expected values in order to better understand the source of the total decay rate enhancements. The expected new QY for each dye can be calculated via Equation (3.16). Values for the radiative decay rate enhancement γ_r/γ_0 and non-radiative decay rate enhancement γ_{nr}/γ_0 are taken from Figure 9.5. For the following analysis, we use $\gamma_{rPB}/\gamma_0 = 2.9$, $\gamma_{nrPB}/\gamma_0 = 1.35$, $\gamma_{rPO}/\gamma_0 = 2.2$ and $\gamma_{nrPO}/\gamma_0 = 1.2$. The intrinsic quantum yield for PB is $\eta_{0PB} = 0.78$ and for PO can be estimated as $\eta_{0PO} \approx 0.5$, respectively [152, 153]. From Equation (3.16), we then arrive at new values for the QYs of $\eta_{PB} = 0.65$ and $\eta_{PO} = 0.56$ when interacting with the tri-disk antenna. This yields QY enhancement factors of $f_{\eta PB} = 0.83$ and $f_{\eta PO} = 1.13$. Both of these enhancement factors are near unity. f_E , however, is ≈ 20 (see Figure 9.4 c)) for the largest part of the field distribution for both dyes, as they are under the same illumination fields inside the antenna. We are, therefore, in a f_E dominated regime as $f_E \gg f_\eta$. This is supported by the observed S values in Figure 9.4 a) and b) ($S_{PB} \approx 16$, $S_{PO} \approx 13$) which are in line with the values attained in the field mapping (see Figure 9.4 a) and b)). Because of this the fluorescent enhancement values in Figure 9.4 a) and b) are directly related to the electric field enhancement of the antenna and not combined with a large QY enhancement factor as would be the case for a low intrinsic QY dye [46, 154].

We can therefore conclude that although plasmonic coupling produces misleading localization results, the resulting emission enhancement has nearly no effect on the total number of collected photons when employing high QY dyes [3, 46]. For this reason, we get similar fluorescence gain values for both dyes, even for PO whose emission is not in

resonance with the plasmonic antenna. Because of this we would expect the total fluorescence collected for each dye to be similar as in both cases the illumination is the same. However, as we noted earlier the light collected for PB is ~3X that of PO.

Using equations (3.7) and (3.13) and applying them to (3.9) then taking the ratio for the total enhanced fluorescent for PB and PO dyes we can write:

$$\frac{F_{enPB}}{F_{enPO}} = \frac{\eta_{PB}}{\eta_{PO}} \frac{\eta_{0PO}}{\eta_{0PB}} \frac{|E|^2}{|E_0|^2} \frac{A}{A} \frac{|E_0|^2}{|E|^2} \frac{\eta_{detPB}}{\eta_{detPO}} \frac{\eta_{0PB}}{\eta_{0PO}} \frac{\sigma_{absPB}}{\sigma_{absPO}} \quad (9.1)$$

As we are using the same plasmonic system for both dyes we can cancel the electric field terms, thus arriving at:

$$\frac{F_{enPB}}{F_{enPO}} = \frac{\eta_{PB}}{\eta_{PO}} \frac{\eta_{detPB}}{\eta_{detPO}} \frac{\sigma_{absPB}}{\sigma_{absPO}} \quad (9.2)$$

Inserting the values for the QYs we calculated from equation (3.16) we obtain $\eta_{PB}/\eta_{PO} = 1.15$. For our optical setup the largest contribution to $\eta_{detPB}/\eta_{detPO}$ is the ratio between the QY of the EMCCD camera at 450nm and 575nm; 0.85 and 0.95, respectively. This yields $\eta_{detPB}/\eta_{detPO} \approx 0.89$. Combining these results, the difference between fluorescence intensity for each dye comes almost entirely from the change in σ_{abs} . The total number of collected photons is, from Figure 9.4 a) and 9.4 b), $F_{enPB} \approx 3000 > F_{enPO} \approx 900$. The ratio of these values is in agreement with that obtained from the literature values of the absorption coefficients $\sigma_{absPB} \approx 46000 \text{ M}^{-1}\text{cm}^{-1}$ and $\sigma_{absPO} \approx 25000 \text{ M}^{-1}\text{cm}^{-1}$ [152]. σ_{abs} for single molecules is slightly modified when adsorbed on the antenna, which likely produces the small deviations we see in our results [155].

Through this analysis, we have shown that the increased LDOS presented by the plasmonic structure does not affect the gain values significantly due to the high initial QY of PB and PO. However, the coupling to the plasmonic modes of the system still prohibits the correct localization of PB (i.e. it affects the spatial location of the emission, without affecting the quantum efficiency of the emitter itself). Because the enhancement of the QY for PO is near unity, the gain values measured predominantly reflect the EM field enhancement, thus our super-resolution localization maps represent a more accurate optical mapping of the EM field around a plasmonic antenna.

10. Conclusion

The initial goals and objectives set out at the early stages of this project have been met. A functioning localization based super-resolution microscope for probing the near-field of plasmonic structures was designed and constructed. TIR illumination was used to excite fluorescence from dye molecules in films and individually as single molecules. We collected fluorescence and were then able to localize the position of single molecules using the maximum likelihood estimation method. This technique was applied to mapping enhancement in plasmonic structures.

Test plasmonic structures of a basic design were then fabricated in gold, via electron beam lithography. These structures were then characterised with scanning electron microscopy to confirm their structure and dark field microscopy to confirm their resonance. These results were compared with simulations of the antennas expected scattering resonances. The antenna were then used to demonstrate fluorescence enhancement for Rhodamine 800 molecules immobilized on a poly-L-lysine film over plasmonic antenna illuminated in TIR with a 640nm CW laser.

Point accumulation imaging in nanoscale technique was demonstrated for fluorescent molecules on rough gold films - displaying bright fluorescent bursts that were used to build up localization distributions for hotspots generated on the illuminated film's surface. We then studied plasmonic bar antenna, which were compared with simulations that calculated expected EM enhancement field maps for the antenna. Initial SR maps for simple antenna compared well with simulated EM field maps, however, these initial experiments did not consider any simulations of the effect of plasmonic system on the emission enhancement of the dye via the changes in the local density of states around the antenna.

The same technique was then used to produce localization distributions for simple plasmonic structures that demonstrated the ability to map and distinguish closely spaced hotspot excitations –two hotspots spaced by 300nm were clearly distinguished.

Antennas featuring a polarization dependent hotspot were then considered. The antennas were simulated and designed to ensure they had demonstrated a clear hotspot switching between polarizations. Although a switchable distribution was observed with an Al dolmen structure, the localization fields seem to fail to represent the EM near-fields structure expected from basic theory and simulation – rather than observing strong gap enhancements, the structures showed enhanced fields centred over individual bars of the dolmen structure. These maps showed the distinguishing of features well below the diffraction limit of a conventional optical system. This mis-localization arises due to fluorescent emission coupling to plasmonic structure.

In order to circumvent this issue, dyes with large Stokes shifts were explored. By decoupling the absorption and emission processes using a large Stokes shift dye, the ability to minimize plasmonic coupling was demonstrated. This removes a major source of error in the localization of single-molecule events when performing super-resolution localization fluorescence microscopy in plasmonic systems. Activating different hotspots in the antenna by changing the polarization allowed us to show that the output data contained in the fluorescence enhancement maps is the combinations of the EM field (polarization sensitive) and the increased LDOS accessible by the molecule (which is independent of the polarization of the illuminating light). By employing a large Stokes-shifted emitter the number of states available to couple into was minimized. This enabled significantly more accurate mapping of the enhanced EM fields alone than has been possible to date. Moreover, the use of high QY fluorescent dyes produced a situation where the plasmonic coupling can affect their apparent emission position, and hence localization, without the increase LDOS, producing a strong enhancement to the total emission. This enabled the easy linking of the fluorescence enhancement values with the EM field enhancement only.

Specifically, Pacific Orange, was used to reduce emission coupling with the plasmonic resonance to reduce the effect of fluorescent molecule mis-localization. Al tri-disk antenna which were resonant with the excitation wavelength of PO were designed and fabricated. Extensive simulations of the properties of the antenna, including EM

distributions from TIR illumination simulations and detailed studies of the decay rate enhancements covering both radiative and non-radiative components, were still required in order to fully understand the observed enhancement factors. The results were compared to the localization fields produced for a small Stokes shifted, emission coupled, Pacific Blue dye. Pacific Orange dye shows a more significant response to the changing EM field distribution, through selective polarization illumination, and gives a better representation of the near-field structure. This enabled us to probe plasmonic near-fields of individual structures experimentally with nm resolution with a greatly reduced effect of fluorescent molecule mis-localization while still exciting strong EM field enhancements with our illumination light.

Although attempts to tune the plasmonic system with respect to the dye molecules emission and excitation response has been made, this pairing of strong illumination enhancement leading, to high absorption enhancement for the dye molecule, and decoupling the emission of the dye molecule from the plasmonic modes of the antenna has not, to our knowledge been previously achieved, making our technique a first for localization based near-field imaging of plasmonic structures. In addition to this, the application of sample tracking and stabilisation allowed the results to be taken from single plasmonic antenna where many attempts have required data taken from multiple antenna to build a single profile [21].

It's expected that this work will encourage new and interesting developments in both the fields of plasmonic and near-field imaging and also in the understanding of the interplay between plasmonic systems and coupled emitters.

11. Future Work

Future work is considered from two different points of view – those that improve the experimental capabilities of the SR system, and those that look at applying the SR microscope to the study of new plasmonic systems.

To push work in any area of science forwards it is necessary to understand the failings and short comings of techniques. Here then we will discuss some of the difficulties faced in attempting to probe EM fields of plasmonic antenna and possible solutions. We will start focusing on the specific experimental work contained in this document but then move to a broader discussion of near-field probing techniques using fluorescent probes.

To prevent fluorescent emission saturation a low illumination power density was used throughout this work. The power for each experimental run was selected by introducing fluorescent molecules at a low concentration to the sample surface, illuminating them at a low power and then gradually increasing the power until regular bursts of fluorescence were observed at antenna locations. For the current fibre coupled to a Nikon TIR illuminator this minimum required power is near the total available power from our laser + fibre system. The combination of using our laser at maximum power combined with field enhancement in gap regions enabled this work to succeed. The small total fluorescent enhancement from simple single component antenna, such as individual bars and individual disks, means that these simpler components cannot be easily mapped and studied as we do not have the available power or signal to noise ratio to detect these interactions. This makes a more basic study of the localization properties and plasmonic fluorescent interactions very difficult. Following this, making extensive use of new large Stokes shifted dye to make more thorough investigations of decoupling effects and how they can improve near-field sensing techniques. For further work in this area we wait upon the development for a more diverse selection of large Stoke shifted fluorescent molecule.

In addition, because of the poor matching of illuminator and microscope it is difficult to achieve high quality TIR illumination as the settings needed are often at the extremity of adjustability of the system. This leads sometimes to poor illumination quality. A

reconstructed illumination system, from the laser to the optics would greatly enhance the capabilities and throughput of this system. The poor power coupling due to fibre and TIR illuminator could be corrected with the use of a direct laser coupling to the microscope without fibre or illuminator. This would allow higher power transfer but also greater control over illumination systems and power densities at the sample surface.

With this implemented future work on more basic localization projects would be possible. A large investigation into more dyes, including larger Stokes shifted dyes, would allow us to solidify knowledge of fluorescent coupling to plasmonic antennas and its effect on localization. It will also allow the system to be used with a much wider variety of plasmonic systems. To make a new technique to a robust and useful addition to common characterisation extensive use of it must be made to understand drawbacks and difficulties that it may face with the plethora of plasmonic systems that are fabricated and designed for diverse applications and make appropriate improvements to the setup.

These could include the study and application of self-assembled monolayers to act as temporary spacer layers, that can be removed without damaging samples, enabling the SR technique to be applied to antenna that do not have to be specifically fabricated to be compatible with the current practice. Development of a technique compatible with supercontinuum illumination systems to give increased flexibility and allow detailed study of how illumination wavelengths effect the localization distributions taken for specific antennas. Improvements to mechanical stability of the system via enhancement of the tracking algorithm and removal of fan assisted cooling in the monitoring cameras. It would also be important to improve the user experience of the setup that could involve chip carriers that allow the mounting of samples without the use of adhesive tape and microfluidic systems to supply the liquid medium and dyes.

An investigation into the use of small quantum dots (QDs) as a probes for a PAINT style near-field mapping could yield extremely beneficial results such as high photo stability and a diverse range of spectral emission and absorption properties. Where fluorescent molecules often only have a relatively small spectral window to excite them at leading

to a restriction in the possible dyes for each antenna design, many QD have extremely spectrally broad absorption profiles, with emissions that can be far into the near infrared. This would make it possible to use the same QD's for almost all optical antennas with sufficient separation between the illumination wavelength and the resonances of the plasmonic antenna, and the emission wavelength of the QD to achieve decoupling of the emitted fluorescence [156-158].

Some preliminary work was done in this area when working on the Al dolmen structure. Achieving the transient bursts of fluorescence that happens with fluorescent molecules as a product of desorption or bleaching proved to be non-trivial. The long photostability of QD [159, 160], which prevents bleaching, and their commonly charged surfaces make interactions between surface and QD strong, in turn making achieving the desired surface action difficult.

Alternate possible solutions for this are to abandon trying to achieve a temporary absorption but instead using a flow chamber to control QD positions. This was demonstrated in a Ropp, C. *et al* paper [161] and shows promise. However using microfluidics to control the QD adds an extra layer of complexity over simply relying on Brownian motion, making it less attractive as a final solution.

Another possible strategy would use QD/molecules bound to the surface permanently and rely on the natural or forced blinking of the QDs to achieve temporally spaced single QD fluorescence which could be localised. This forms the basis of the PALM/STORM style technique used for fluorescent dye molecules and has been demonstrated to work for plasmonic structures [90]. It has also been partially demonstrated with PALM/STORM style QD mapping of biological structures [162, 163] and shown to be possible for large scale silver nanowires [92]. This technique shows promise but the extremely high QD density necessary to probe the near-field structure of a lot of small scale plasmonic antenna may prove to be an insurmountable barrier for probing single antenna.

Recent work on using emitters fixed to the end of AFM tips [93-96] have allowed the study of fluorescence lifetime and enhancement factors of the QDs as the AFM tip is scanned over a silver nanowire. These scanning based tip based techniques have many

advantages over PAINT based techniques. For one localization is not needed as the position of the tip is known therefore the fluorescence collected can be attributed to the tips current location. It also means that the investigator is in full control of the fluorescent probes location making in-depth studies of specific locations around a plasmonic system possible. The techniques, however, suffer due to the difficulty in fabricating this type of probe but also the photobleaching of fluorescent molecule or QD on the probe. Lastly the introduction of the probe itself will affect the plasmonic system's response.

Returning to the discussion of PAINT based near-field mapping once many of the initial problems have been address possible future project may involve trying to bring more of the techniques that have already been applied in SR localization microscope to bear on the problems of PAINT basted SR microscopy.

3D localization has been applied to great success in biological imaging and the techniques used for this would be highly suited to allow SR near-field mapping to be performed on some of the interesting 3D plasmonic structures generated by techniques such as multiphoton direct laser lithography.

Multi-colour fluorescence mapping allows higher than normal probe density in PALM and STORM microscopy as each type can be individually filtered out from the others to maintain spatial separation between individually active fluorescent molecules. This same idea could easily be applied to PAINT based SR near-field microscopy. It would allow for the simulations probing of multiple different emission wavelengths as each dye could be individually spectrally selected with specific optical filters.

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Appendix A

Initial Sort Function

```
function
[array_Imnum,photon_number,image]=Linitialsort(XYlist,start
,imnumber,boxsize,bgboxsize,photonstepsize,numberofphotonst
eps)
s=size(XYlist);
[filename,PathName,FilterIndex]=uigetfile;
array_Imnum = zeros(s(1),imnumber,numberofphotonsteps);
photon_number= zeros(s(1),imnumber);
XYminlist=zeros(s(1),s(2));
XYminlist2=zeros(s(1),s(2));
D = boxsize/2;
DD= bgboxsize/2;
rect=zeros(s(1),4);
rect2=zeros(s(1),4);
C = imread([PathName filename]);
D2=size(C);
A2=zeros(D2(1),D2(2));
A2=uint64(A2);
for e = 1:s(1)
    XYminlist(e,1)=XYlist(e,1)-D;
    XYminlist(e,2)=XYlist(e,2)-D;
    rect(e,:) = [XYminlist(e,1),XYminlist(e,2),boxsize-
1,boxsize-1];
end
for f = 1:s(1)
    XYminlist2(f,1)=XYlist(f,1)-DD;
    XYminlist2(f,2)=XYlist(f,2)-DD;
    rect2(f,:)=[XYminlist2(f,1),XYminlist2(f,2),bgboxsize-
1,bgboxsize-1];
end
```

```

mask=ones(boxsize,boxsize);
mask((bgboxsize/2)+boxsize/2,(bgboxsize/2)+boxsize/2)=0;
mask=rot90(mask);
mask=rot90(mask);
mask(bgboxsize,bgboxsize)=0;
mask=not(mask);
mask=uint16(mask);
h = waitbar(0,'Initializing waitbar...');
for k = start:(imnumber+start)
    if k-1 < 10
        % T = ([PathName 'img_00000000' num2str(k-1)
'_Default_000.tif']);
        % T = ([PathName 'img_00000000' num2str(k-1)
'__000.tif']);
        T =([PathName '0 (' num2str(k) ').tif']);
    end
    if k-1 > 9
        if k-1 < 100
            % T = ([PathName 'img_00000000' num2str(k-1)
'_Default_000.tif']);
            %T = ([PathName 'img_00000000' num2str(k-1)
'__000.tif']);
            T =([PathName '0 (' num2str(k) ').tif']);
        end
    end
    if k-1 >999
        if k <10000
            % T = ([PathName 'img_000000' num2str(k-1)
'_Default_000.tif']);
            %T = ([PathName 'img_000000' num2str(k-1)
'__000.tif']);
            T =([PathName '0 (' num2str(k) ').tif']);
        end
    end
end

```

```

end
if k-1 > 9999
    if k-1 <100000
        % T = ([PathName 'img_0000' num2str(k-1)
'_Default_000.tif']);
        %T = ([PathName 'img_0000' num2str(k-1)
'__000.tif']);
        T =([PathName '0 (' num2str(k) ').tif']);
    end
end
A = imread(T);
for g = 1:s(1)
    PSFcrop=imcrop(A,rect(g,:));
    BGcrop=imcrop(A,rect2(g,:));
    BGcrop=uint16(BGcrop);
    BGcrop=mask.*BGcrop;
    bg=sum(sum(sum(BGcrop)))/( (bgboxsize*bgboxsize) -
(boxsize*boxsize));
    Av = meanabs(PSFcrop);
    N = (Av-bg)*boxsize*boxsize;
    photonselect=N/photonstepsize;
    photonselect=ceil(photonselect);
    if photonselect-1>numberofphotonsteps
        array_Imnum(g,k-start+1,:)=1;
    else
        array_Imnum(g,k-start+1,[1:photonselect-1])=1;
    end
    photon_number(g,k-start+1)=N;
    %for p = 1:ps(1)
    %     if N > (photonnumber(p))
    %         array_Imnum(g,k,p)=1;
    %         photon_number(g,k)=N;
    %     end

```

```

        %end
        A=uint64(A);
        A2= A2+A;
    end
    mes = (['# ' num2str(k)]);
    waitbar(k/imnumber,h,mes);
end
image=A2;
end

```

Sequence Localization Function

```

function
[result]=LLocalizeM(XYlist,r,photoncrop,ROIcrop,sortedstack
,imnumber,startnumber)
s = sum(sortedstack,2);
s2=max(s);
s3=size(XYlist);
imnum = zeros(s3(1),s2);
array_g = zeros(5,s2,s3(1));
array_gv = zeros(s3(1),s2);
[filename,PathName,FilterIndex]=uigetfile;
%PathName='C:\Users\dlm08\Documents\Work\PhD\151023 H#001
BEAST A1 antenna 405\151024_0418_1\Pos0\';
%preping cropping data for photon# estimate
XYminlist=zeros(s3(1),s3(2));
D = photoncrop/2;
rect=zeros(s3(1),4);
for h = 1:s3(1)
    XYminlist(h,1)=XYlist(h,1)-D;
    XYminlist(h,2)=XYlist(h,2)-D;
    rect(h,:) =
[XYminlist(h,1),XYminlist(h,2),photoncrop,photoncrop];
end

```

```

%preping cropping data for ROI
XYminROIlist=zeros(s3(1),s3(2));
D2 = ROIcrop/2;
rectROI=zeros(s3(1),4);
for g = 1:s3(1)
    XYminROIlist(g,1)=XYlist(g,1)-D2;
    XYminROIlist(g,2)=XYlist(g,2)-D2;
    rectROI(g,:) =
[XYminROIlist(g,1),XYminROIlist(g,2),ROIcrop,ROIcrop];
end
XYcenter=[(ROIcrop/2)*39.5,(ROIcrop/2)*39.5];
%ready image number counter
M=ones(1,s3(1));
%create stack check variable
check=sum(sortedstack);

%ready diplay bar.
h = waitbar(0,'Initializing waitbar...');
%main loop
for k = startnumber:(imnumber+startnumber)
    %is there any flashes at all in this image?
    if check(k-startnumber+1) >= 0.5
        %load image number k
        if k-1 < 10
            % T = ([PathName 'img_00000000' num2str(k-1)
'_Default_000.tif']);
            % T = ([PathName 'img_00000000' num2str(k-1)
'__000.tif']);
            T =([PathName '0 (' num2str(k) ').tif']);
        end
        if k-1 > 9
            if k-1 < 100

```

```

        % T = ([PathName 'img_0000000' num2str(k-1)
'_Default_000.tif']);
        %T = ([PathName 'img_0000000' num2str(k-1)
'__000.tif']);
        T =([PathName '0 (' num2str(k) ').tif']);
    end
end
if k-1 > 99
    if k-1 < 1000
        %T = ([PathName 'img_000000' num2str(k-1)
'_Default_000.tif']);
        % T = ([PathName 'img_000000' num2str(k-1)
'__000.tif']);
        T =([PathName '0 (' num2str(k) ').tif']);
    end
end
if k-1 >999
    if k-1 <10000
        %T = ([PathName 'img_00000' num2str(k-1)
'_Default_000.tif']);
        %T = ([PathName 'img_00000' num2str(k-1)
'__000.tif']);
        T =([PathName '0 (' num2str(k) ').tif']);
    end
end
if k-1 > 9999
    if k-1 <100000
        % T = ([PathName 'img_0000' num2str(k-1)
'_Default_000.tif']);
        %T = ([PathName 'img_0000' num2str(k-1)
'__000.tif']);
        T =([PathName '0 (' num2str(k) ').tif']);
    end
end

```

```

end
A = imread(T);
    %get BG for this image
%bg=0.9;
bg = meanabs(A);
    %checking each seperate point on image
for e=1:s3(1)
    %is there a at this coordiante on
this image?
    if sortedstack(e,k-startnumber+1) >= 0.5
        %calculate photon
estimation
        B=imcrop(A,rect(e,:));
        Av = meanabs(B);
        N = (Av-bg)*photoncrop*photoncrop;
        %N=300;
        %do ROI image crop
        C=imcrop(A,rectROI(e,:));
        %update initial parameters
        %           X           Y           r   pix
        params =
[XYcenter(1),XYcenter(2),r,(bg^0.5),N];

        %run MLE for this location
        [g,gv] = MLEwG(C,params,39.5,0,1,0);
        %update stored variables
        array_g(:,M(e),e)=g;
        array_gv(e,M(e))=gv;
        %add image number to
imnumlist and iterate M for this
        %location
        imnum(e,M(e))=k;
        M(e)=M(e)+1;

```

```

        else
        end
    end
    %update display bar
    mes = (['# ' num2str(k)]);
    waitbar((k-startnumber)/imnumber,h,mes);
end
end
result=Lcombine(array_g,array_gv,imnum);

```

Combine Result Function

```

function
[resultscombi]=Lcombine(array_g,array_vg,array_imnum)
s = size(array_g);
array_vg=array_vg';
array_imnum=array_imnum';
resultscombi = zeros(s(2),7,s(3));
array_g2=zeros(s(2),s(1),s(3));
for l = 1:s(3)
    array_g2(:, :, l)=array_g(:, :, l)';
end
for k = 1:s(3)
    resultscombi(:, :, k)=[array_imnum(:, k),array_g2(:, 1, k),array
_g2(:, 2, k),array_g2(:, 3, k),array_g2(:, 4, k),array_g2(:, 5, k),
array_vg(:, k)];
end

```

Temporary Plot Function

```

function Ltempplot(Result)
s=size(Result);
for k=1:s(1)
    if Result(k,1)==0
        break
    else

```

```

        Temp(k,:) = Result(k,:);
    end
end
figure(1)
scatter3(Temp(:,2),Temp(:,3),Temp(:,6),5,Temp(:,6));
title('3d Scatter Plot')
figure(2)
scatter(Temp(:,2),Temp(:,6),5,Temp(:,6));
title('Xproject Scatter Plot')
figure(3)
scatter(Temp(:,3),Temp(:,6),5,Temp(:,6));
title('Yproject Scatter Plot')
end

```

Variance Fitting Filter

```

function
[Datastackout]=Lvarfilter(Datastack,Yo,A,t,varmax,varmin)
S=size(Datastack);
M=1;
for k = 1:S(1)
    if Datastack(k,7) < (Yo+A*exp(-Datastack(k,6)/t))
        if Datastack(k,7)<varmax
            if Datastack(k,7)>varmin
                Datastackout(M,:)=Datastack(k,:);
                M=M+1;
            end
        end
    end
end
end

```

Spurious Localization Filter

```

function
[fdata]=Lfilter(data,XYlist,xmax,xmin,ymax,ymin,varmax,varmin,photonmax,photonmin,diametermax,diametermin)

```


Appendix B

Far-field Projection

```
#####
# User Settings
NA = 1;          # choose NA of imaging optics
mname = "Monitor1"; # monitor recording the data
res = 1001;      # res controls the number of plane waves
                  # used for decomposition, which is (res x
res)
z0 = 0e-6;      # defocus
###
xmax=2000e-9;
xmin=-2000e-9;
ymax=2000e-9;
ymin=-2000e-9;
#####

f = getdata(mname,"f"); # frequency of monitor
x=getdata(mname,"x");
y=getdata(mname,"y");
lamda=c/f;             # wavelength
k=2*pi/lamda;          # k
EM=getelectric("1nm_mon");
stuffx=getdata("1nm_mon","x");
stuffy=getdata("1nm_mon","y");
write("160607_1213_xvalues.txt",num2str(stuffx));
write("160607_1213_yvalues.txt",num2str(stuffy));

for(j=1:1:33)
{
StringPosition=num2str(j);
Filename="160607_1213_lambda"+StringPosition+"_25+375.txt";
Filenameim="EM160607_1213_lambda"+StringPosition+"_25+375.txt";

# decompose nearfield into plane waves using farfield projection
E = farfieldvector3d(mname,j,res,res);
Ex = pinch(E,3,1);
Ey = pinch(E,3,2);
Ez = pinch(E,3,3);
ux = farfieldux(mname,j,res,res);
uy = farfielduy(mname,j,res,res);
Ux = meshgridx(ux,uy);
Uy = meshgridy(ux,uy);
Uz = real(sqrt(1-(Ux^2+Uy^2))) + 1e-20;

# filter for propagating waves through aperture
filter = real(sqrt(Ux^2 + Uy^2)) < NA;
Ex=filter*Ex;
Ey=filter*Ey;
Ez=filter*Ez;

# define image plane
#x_image=linspace(min(x),max(x),res);
```

```

#y_image=linspace(min(y),max(y),res);
x_image=linspace(xmin,xmax,res);
y_image=linspace(ymin,ymax,res);

# calculate the image, using chirped z-transform
kx = ux*k(j);
ky = uy*k(j);
dkx = kx(2)-kx(1);
dky = ky(2)-ky(1);
Kz = Uz*k(j);

Ex_image =
czt(Ex*exp(1i*Kz*z0)*sqrt(1/Uz/k(j)^2),kx,ky,x_image,y_image)*dkx*d
ky/(2*pi);
Ey_image =
czt(Ey*exp(1i*Kz*z0)*sqrt(1/Uz/k(j)^2),kx,ky,x_image,y_image)*dkx*d
ky/(2*pi);
Ez_image =
czt(Ez*exp(1i*Kz*z0)*sqrt(1/Uz/k(j)^2),kx,ky,x_image,y_image)*dkx*d
ky/(2*pi);

# calculate |E|^2 at the image plane
E2_image=abs(Ex_image)^2 + abs(Ey_image)^2 + abs(Ez_image)^2;
write(Filename,num2str(E2_image));
Etemp=pinch(pinch(EM,4,j));
write(Filenameim,num2str(Etemp));
}

```

Dipole Iteration

```

#####
##
# Scriptfile: Flourescence_LDOS_scan.lsf
#
# Description: This file can be used to setup decay rate
# calculations for a dipole scanned over a set
# objects.
#####
##

#####
##
# USER ENTERED VALUES
#####
##
#Structure Variables
Material="Al (Aluminium) - Palik Copy 1";
eps_r=1.52;
#####
##
#Simulation Parameters
#####
##
#Dipole Scan Variables
Xscanmin=0;
Xscanmax=0;

```

```

Xintervalnumber=1;      #for nice step size of a Xintervalnumber =
                        #                               ((Xscanmax-
Xscanmin)/a)+1
Yscanmin=-100 ;
Yscanmax=100;
Yintervalnumber=41;      #for nice step size of a Yintervalnumber =
                        #                               ((Yscanmax-
Yscanmin)/a)+1
Hight=25e-9;            #Hight of sweep above 0
polarisation=1;         #0 x 1 y 2 z set this the same as simulation
#####
##
#Filesetup
Filename="160206_1353_tridiskD70G30_DMSO_polay_X";
Fileend=".txt";
#####
##
#####
##
#####
##
#####
##
#####
##
#####
##
#    scan
#####
##
XStepsize=(Xscanmax-Xscanmin)/(Xintervalnumber-1);
YStepsize=(Yscanmax-Yscanmin)/(Yintervalnumber-1);
total=Xintervalnumber*Yintervalnumber;
count=0;
for(i=Xscanmin:XStepsize:Xscanmax)
{
for(j=Yscanmin:YStepsize:Yscanmax)
{
Xposition=num2str(i);
Yposition=num2str(j);
select("source1");
set("z", (Hight));
set("x", (i*1e-9));
set("y", (j*1e-9));####move source1
select('QE');
set('dipole x', (i*1e-9));
set('dipole y', (j*1e-9));
set('dipole z', (Hight));####move dipole monitor box
run;
Xtest=pinch(getdata("source1","x"));
Ytest=pinch(getdata("source1","y"));
Ztest=pinch(getdata("source1","z"));
count=count+1;
?"X="+num2str(Xtest*1e9)+"nm Y="+num2str(Ytest*1e9)+"nm
Z="+num2str(Ztest*1e9)+ " %="+num2str((count/total)*100);
monitors;
select('QE');
set('use dipole box',1);

```

```

runanalysis('QE');
def=getdata('QE','f');
gamma_radiative=getdata('QE','gamma_radiative');
gamma_non_radiative=getdata('QE','gamma_nonradiative');
QE_fdttd=getdata('QE','QE_fdttd');
runanalysis('trans_box');
if (polarisation==0)
{
component = "Ex0";
}
if (polarisation==1)
{
component = "Ey0";
}
if (polarisation==2)
{
component = "Ez0";
}
f=getdata("trans_box","f");
E = pinch(getdata("source1",component));
t = getdata("source1","time0");
E_w = czt(E,t,2*pi*f)*(t(2)-t(1));
moment = getnamed("source1","total amplitude");
m = moment*getdata("source1","time_signal");
ts = getdata("source1","time");
m_w = czt(m,ts,2*pi*f)*(ts(2)-ts(1));
w = 2*pi*f;
G=(E_w)*c^2*eps0*eps_r/(w^2*m_w);
rho_z= [2*w/pi/c^2]*imag(G);
gammasimulated= pi*w/hbar/eps0*1^2*rho_z;
gammanumerical=((w^3)*(1^2))/(3*pi*eps0*hbar*(c^3));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_DEF"+Fileend;
write(File,num2str(def));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_DEgamma_radiative"+Fileend;
write(File,num2str(gamma_radiative));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_DEgamma_non_radiative"+Fileend;
write(File,num2str(gamma_non_radiative));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_DEQE"+Fileend;
write(File,num2str(QE_fdttd));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_LDOSimag(G)"+Fileend;
write(File,num2str(imag(G)));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_LDOSgammasimulated"+Fileend;
write(File,num2str(gammasimulated));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_LDOSgammanumerical_freespace"+Fileend;
write(File,num2str(gammanumerical));
File=Filename+Xposition+"nm_Y"+Yposition+"nm_LDOSgammasim_over_gammanumerical_freespace"+Fileend;
write(File,num2str(gammasimulated/gammanumerical));
switchtolayout;
}
}

```