NOVEL FABRICATION AND TESTING OF LIGHT CONFINEMENT DEVICES

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## 2 Zero mode waveguides

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ABSTRACT

Novel fabrication and testing of light confinement devices
A thesis submitted to The University of Manchester
for the degree of
doctor of philosophy
(PhD)
by Josh Ring
July 18, 2016

The goal of this project is to study novel nanoscale excitation volumes, sensitive enough to study individual chromophores and go on to study new and exciting self assembly approaches to this problem. Small excitation volumes may be engineered using light confinement inside apertures in metal films. These apertures enhance fluorescence emission rates, quantum yields, decrease fluorescence quenching, enable higher signal-to-noise ratios and allow higher concentration single chromophore fluorescence, to be studied by restricting this excitation volume. Excitation volumes are reported on using the chromophore’s fluorescence by utilising fluorescence correlation spectroscopy, which monitors fluctuations in fluorescence intensity. From the correlation in time, we can find the residence time, the number of chromophores, the volume in which they are diffusing and therefore the fluorescence emission efficiency. Fluorescence properties are a probe of the local environment, a particularly powerful tool due to the high brightness (quantum yield) fluorescent dyes and sensitive photo-detection equipment both of which are readily available, (such as avalanche photodiodes and photomultiplier tubes). Novel materials combining the properties of conducting and non-conducting materials at scales much smaller than the incident wavelength are known as meta-materials. These allow combinations of properties not usually possible in natural materials at optical frequencies. The properties reported so far include; negative refraction, negative phase velocity, fluorescence emission enhancement, lensing and therefore light confinement has also been proposed to be possible. Instead of expensive and slow lithography methods many of these materials may be fabricated with self assembly techniques, which are truly nanoscopic and otherwise inaccessible with even the most sophisticated equipment.

It was found that nanoscaled volumes from ZMW and HMMs based on NW arrays were all inefficient at enhancing fluorescence. The primary cause was the reduced fluorescence lifetime reducing the fluorescence efficiency, which runs contrary to some commentators in the literature. NW based lensing was found to possible in the blue region of the optical spectrum in a HMM, without the background fluorescence normally associated with a PAA template. This was achieved using a pseudo-ordered array of relatively large nanowires with a period just smaller than $\lambda/2$ which minimised losses. Nanowires in the traditional regime $\lambda/10$ produced significant scattering and lead to diffraction, such that they were wholly unsuitable for an optical lensing application.
DECLARATION

The University of Manchester

Candidate Name: Josh Ring

Faculty: Engineering and Physical Sciences

Thesis Title: Novel fabrication and testing of light confinement devices

Declaration to be completed by the candidate:

I declare that no portion of this work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Signed: Date: July 18, 2016
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ACKNOWLEDGEMENTS

This thesis was not created alone, I would like to thank Eric Whittaker for his patient critical analysis and discussion. My supervisors Dr. Alex Jones and Bruce Hamilton for supporting my stay in Manchester after my group disbanded, through frequent meetings and encouragement.

I would like to thank Scott Lewis and Mal McGovern for their informed advice about micro and nanofabrication.
CHAPTER ONE

INTRODUCTION
1.1 Aims and objectives

To create a light confinement device which is compatible with conventional microscopes and samples. Once devices have been constructed and their structure has been analysed, they will be optically characterised, compared to conventional microscopy as a control. Two classes of device will be discussed in this work, focusing and non-focusing. These operate by different mechanisms, but both aim to achieve light confinement.

1.2 General introduction

The goal of this thesis is to advance the current state of microscopy by using novel materials to restrict light to nanoscopic volumes. This work contrasts many of the next generation microscopy methods, by not requiring sample modifications to visualise the structure or complex excitation schemes, which limit the number of applications they can be used for.

Inside the light emitting regions of molecules known as chromophores, the electrons may be excited by light. Upon returning to their original energy state emit the difference in energy as light of a lower energy than was input. This is the process of fluorescence emission and is outlined in section 1.3.

Chromophores may be attached to biological samples[1] in order to highlight an area of specific interest, in a process known as fluorescent labelling[1]. Labelling can be highly specific to a given cell structure or macromolecular component, since it can be tailored to the unique chemicals exposed on the structure[1]. This has motivated research to improve the brightness and efficiency of chromophores, which can now be detected individually as is illustrated in section 1.5.

New microscopy methods can also increase the brightness of these chromophores by using light confinement inside metal structures. The simplest example of this is a nanoscopic hole in a thin metal film, which when illuminated from below creates a small excitation volume inside the hole[2, 3]. The metal’s surface electrons are excited by the light and create their own local electric field in addition to the incident light as outlined in section 1.9. The combination of light and electron excitation has been reported to
enhance fluorescence emission rates\cite{4, 5} and fluorescence quantum yields\cite{6} and decrease fluorescence quenching\cite{6}. This enables fluorescence measurements to be made with higher signal-to-noise ratios\cite{7}. Light confinement in metal structures allow individual chromophores to be spatially isolated at concentrations factors of 10–100 higher\cite{2, 3, 8} these devices are detailed in section 2.

The size and efficiency of excitation volumes can be investigated, by monitoring the fluorescence intensity as a function of time\cite{9–11}. From the correlation of fluorescence in time, we find the time occupied by the chromophore inside the excitation volume\cite{9–11}. This time correlates to the size of the volume, since the diffusion rates of many chromophores are known\cite{9–11}. The number of chromophores is related to the correlation amplitude, and therefore the emission per chromophore can be calculated\cite{9–11}. This technique is termed fluorescence correlation spectroscopy, and is detailed in section 1.7

Light confinement into nanoscopic volumes could lead to increased resolution, compared to standard microscopy imaging. The conventional limit is called the diffraction limit\cite{12} and with the best optics with blue light can resolve 130 nm, for more details see section 1.4.2.

Meta-materials combine the properties of non-conducting dielectrics and conducting metallic materials, at sizes much smaller than the incident wavelength\cite{13, 14}. This allows combinations of properties not usually possible in natural materials at optical frequencies\cite{15}. The properties reported so far include: negative refraction\cite{16}, negative phase velocity\cite{17}, fluorescence emission enhancement\cite{18}, monochromatic light lensing\cite{15}. The lensing is predicted to rival metal light confinement as a way of concentrating light beyond the diffraction limit\cite{15}. This is explored in sections 1.9.

Instead of expensive and slow lithography methods, meta-materials may be fabricated with self assembly techniques. These techniques are truly nanoscopic, and produce structures which are beyond even the most sophisticated lithography equipment, refer to chapter 3.
1.3 Fluorescence

Fluorescence is a radiative decay process for species which have entered an excited state, such as by the absorption of a photon of energy corresponding to the gap between a pair of energy levels. As shown in figure 1.1, the transitions leading to fluorescence arise mostly from the ground state, lowest vibrational level. This is because that is the most populated level according to the Boltzmann factor\[19\]. The Boltzmann factor is given as,

$$\exp \left( -\frac{E_i}{k_B T} \right)$$

where $E_i$ is the energy at integer energy levels $i$, $k_B$ is the Boltzmann constant, $T$ is the temperature. The Boltzmann factor is proportional to the probability of occupancy in a system, in thermal equilibrium, where the population distribution is temperature dependent. The Boltzmann factor is greatest for the lowest $E_1$ level.

The fluorescence spectrum therefore contains contributions primarily from the excited state’s vibrational structure and this in-turn broadens the distribution of energies. The transition probabilities are also affected by the Franck-Condon principle\[20\]. This principle states that, the degree of overlap between vibrational wavefunctions in the ground and excited state energy levels, determine the probability of the transition.

If the excited chromophore has not become de-excited by chemical reaction or non-radiative decay, which is discussed section 1.3.1, then it can radiatively transition back to the ground state. After the transition, vibrational relaxation redistributes the vibrational energy levels according to the thermal energy level distribution. Vibrational relaxation and electronic reorganisation upon transition gives rise to a red shift in the emitted fluorescence spectrum, compared to the absorption spectrum.
Phosphorescence and fluorescence are both radiative transitions, depicted in figure 1.1, from the excited to the ground state occurring via differing mechanisms. Fluorescence involves no change in electron spin $\Delta S = 0$, and occurs on the femtosecond to microsecond time-scale. Phosphorescence by contrast, occurs via inter-system crossing into a triplet state, which is a $\Delta S = 1$ transition.

The relaxation process from the triplet state is relatively slow, from milliseconds to seconds. Direct transitions to the generally singlet ground state, would give two electrons with the same set of quantum states. Two electrons sharing the same quantum states is forbidden by the Pauli-exclusion principle, so the transition has low probability and explains the slow rate of phosphorescence. The transition may occur by spin-orbit coupling due to the presence of paramagnetic atoms.

Chromophores appear dark while in the triplet state, because of the order of magnitude slower rate of phosphorescence compared to fluorescence. The factors which affect the quantum yield of the system are detailed in the next section.

Figure 1.1: Jablonski energy level diagram showing the electron spin state changes $\Delta S = 1$ associated with phosphorescence shown green, and the unchanged spin states $\Delta S = 0$ of fluorescence shown blue. Where the energy levels are labelled from the bottom up as $E_n$, where $n = 1, 2, 3 \ldots$ energy levels. Vertical transitions refer to the electron energy changes, diagonal lines represent incident or emitted light. The x-axis represents the electron configuration coordinate.
1.3.1 Non-Radiative Decay

The rate of non-radiative decay $k_{\text{non-radiative}}$ and radiative decay rate $k_{\text{radiative}}$ affects the quantum yield $\phi$ of the system according to,

$$\phi = \frac{k_{\text{fluorescence}}}{k_{\text{inter-system crossing}} + k_{\text{vibration}} + k_{\text{energy transfer}} + k_{\text{collision}} + k_{\text{fluorescence}}}$$  \hspace{1cm} (1.3.2)

where $k_{\text{mechanism}}$ is the rate of a given mechanism. These terms may be collected as a single non-radiative decay rate and simplified to,

$$\phi = \frac{k_{\text{fluorescence}}}{k_{\text{non-radiative}} + k_{\text{fluorescence}}}$$  \hspace{1cm} (1.3.3)

Inter-system crossing is promoted by Franck-Condon overlap of vibrational levels, and in the presence of relatively heavy atoms. The transition proceeds more rapidly between two states if the energy difference between the spin-states is minimised.

Vibrational redistribution of energy within the same species can allow some energy dissipation. How much this occurs depends on the number degrees of freedom. Energy transfer is a process where the electronic states of two chromophores have a region of energy overlap, and occurs most efficiently when both dipoles are parallelly aligned. These may transfer energy from one to the other non-radiatively. Collisional de-excitation is where the excited species distributes the excess energy into kinetic energy and collides with surrounding species to distribute the energy.
1.4 Microscopy

1.4.1 Compound microscope

The original microscope was the compound microscope originating from the 1620’s, which had only two optical components[21]. First an objective lens which collected the microscopic detail and projected it into the second component, an enlarging eyepiece with which it was viewed. The microscope design is depicted schematically in figure 1.2.

![Schematic of a compound microscope with the object being imaged shown in red.](image)

Since the original invention there have been many improvements in the optical design and sample illumination. Köler illumination for instance prevents an image of the light source appearing in the image by ensuring the illumination rays are parallel at the sample plane and defocused in the image plane. Recently high numerical aperture (NA), objectives have allowed novel types of microscopy, NA is also critical to the resolution as outlined in section 1.4.2. NA defines the range of angles that light from the sample can enter the objective lens and is given by equation 1.4.1 below,

\[ NA = n \sin(\theta) \] (1.4.1)

where \( n \) is the refractive index of the immersion medium, and \( \theta \) is the sine of the light acceptance angle from the lens surface normal vector as shown in figure 1.3. Example values for immersion media refractive index are \( n_{\text{air}} = 1 \), \( n_{\text{water}} = 1.33 \) and \( n_{\text{oil}} = 1.45 \).
1.4.2 Diffraction limit

The minimum resolvable distance, \( d \) or diffraction limit of a conventional microscope, in the far field is given in equation 1.4.2. It is dependent on the wavelength of light \( \lambda \) and the numerical aperture \( NA \) of the objective lens as described by,

\[
d = \frac{\lambda}{2NA}.
\]  

(1.4.2)

1.4.3 Nyquist limit

In fluorescence microscopy samples are densely labelled with fluorescent dyes, to resolve a feature of length \( d_{\text{feature}} \) the maximum separation between fluorescent dyes in that area \( d_{\text{fluorescent labels}} \) is given by 1.4.3,

\[
d_{\text{feature}} \geq 2d_{\text{fluorescent labels}}
\]  

(1.4.3)

So to resolve a feature one micron across there must be at least two fluorescent labels per micron, more fidelity is gathered by adding more labels up to the fundamental resolution of the microscope.

Due to the smaller length scales involved the Nyquist limit is crucially important in super resolution microscopy, see section 1.6.
1.5 Single Molecule Techniques

Single molecule (SM) analysis is particularly powerful for studying asynchronous systems, where the processes occur at different times. In such a system, changes occurring to a single molecule become averaged among many others when observing a significant portion of the whole mixture at once. Thus only noise induced fluctuations around the mean are observed with such a measurement.

Molecular dynamics observed at the SM level can build a distribution of the measured parameter\[22–24\] instead of just recording the average value. SM measurements such as the distances between energy transfer pairs, which are mapped by changes in their excited state properties, see section 1.8. Distance measurements at the molecular level allow the large scale structures of fluorescently labelled proteins and DNA strands to be mapped over several nanometers.

Other types of measurement which can be done on the SM level include atomic force microscopy[25, 26] to study larger structures such as polymers or proteins, or SM linear force measurements between pairs of optical tweezers[27, 28].

Fluorescence on the SM level is also possible due to the high detection sensitivity of avalanche photodiodes and photomultiplier tubes and brightness of modern chromophores. These signals can be further enhanced using nanoscale metal structures[29] and novel metamaterials as outlined in section 1.9.

For SM observation, discrimination of the signal from the background is essential, this may be achieved temporally or spatially. Spatial discrimination uses a small excitation volume as in confocal microscopy and total internal reflection microscopy[30]. To ensure that only one molecule is excited at once, the concentration is of the order of pico–nano molar.

1.5.1 Total internal reflection microscopy

Total internal reflection microscopy (TIRF), improves contrast by exciting molecules in a thin section 100–300 nm in depth [30] rather than over a 1500–5000 nm depth as in conventional microscopy. The excitation depth is adjustable to ensure high signal intensity by varying the total internal reflection angle as shown in figure 1.4 right.
The incident laser beam totally internally reflects at the glass-sample interface, the imaginary component of the light’s electric field then decays rapidly into the sample. The experimental set up is illustrated in figure 1.4 left. The excitation laser shown blue, is expanded to over-fill the objective, the light is then focused at a sharp angle by the high NA objective onto the glass-sample interface, which is often predominately water based. The fluorescence from TIRF excitation, shown green, is then passed through the dichroic and focused onto the camera. The power of the electric field \( P \) depends on the wave vector \( k \) which defines the decay constant, the axial distance \( z \), the incident angle \( \theta_i \), the refractive index of the microscope slide \( n_2 \) and sample \( n_1 \). according to equations 1.5.1 and 1.5.2.

Coupling efficiency is high only if the incident angle \( \theta_i \) is greater than the critical angle \( \theta_c \) which is shown in equation 1.5.3,

\[
P = P_0 \exp(-2kz) \tag{1.5.1}
\]

where,
\[
k = \frac{\omega}{c} \sqrt{(n_2 \sin \theta_i)^2 - (n_1)^2} \tag{1.5.2}
\]

and,
\[
\theta_c = \sin^{-1}(\frac{n_1}{n_2}) \tag{1.5.3}
\]

where \( \omega \) is the angular frequency of light, \( c \) is the speed of light. \( n_1 \) is the medium inside which light is incident onto the interface of the second medium \( n_2 \). This is illustrated in

Figure 1.4: **Left:** Schematic diagram of objective TIRF microscopy. **Top right:** Schematic of the experimental geometry at the interface when excited at the critical angle, where light is reflected parallel to the interface. **Bottom Right:** Normalised intensity decay curve for TIRF in the axial direction with different incident angles above the critical angle \( \theta_c \) of 64.8°.
1.5. SINGLE MOLECULE TECHNIQUES

For example a 488 nm laser undergoing TIRF through a glass slide \( n = 1.45 \), into a water sample \( n = 1.33 \), at an incident angle of 72\(^\circ\), is illustrated by the green curve in figure 1.4 right. The decay constant \( k \), is \( 5.548 \times 10^6 \) and the excitation depth is 90 nm to \( \frac{1}{e} \) of the starting intensity. The TIRF method excites only a small vertical section, which is well suited to surface studies and can resolve surface dynamics on the SM scale[31]. This sensitivity and contrast improvement has lead to it’s use in super resolution techniques outlined in section 1.6.

### 1.5.2 Confocal microscopy

![Confocal microscopy diagram](image)

Figure 1.5: **Left:** Schematic diagram of confocal microscopy, excitation light is shown blue and fluorescence in green. The high NA objective has a shallow depth of focus, restricting excitation axially. Light in the focal plane but not inside the focal spot is rejected by the confocal pinhole, creating a three dimensional confocal excitation volume. **Right:** Axial and radial confocal volume profiles in red and blue respectively.

Confocal microscopy is a point scanning microscopy illustrated in figure 1.5, which rejects out of focus and scattered light by creating a three dimensional confocal volume. This allows depth sectioning inside samples and three dimensional imaging with improved resolution compared to conventional microscopy.

Due to the small excitation volume of the order of femto-litres confocal microscopy has relatively low signal levels, compared to conventional microscopy. It is however able to obtain SM results with a chromophore concentration of the order of pico-molar. The small excitation volume, contrast improvement from removing the out of focus signal and scattered light has lead to it’s use in super resolution techniques outlined in section 1.6.
1.6  Super resolution microscopy

Super resolution microscopy techniques are the next generation of microscopy techniques, which offer improved resolution down to 30–80 nm depending on the technique. They suffer from the very difficult alignment of multiple lasers, and the high cost of equipment and in some cases sample preparation where genetic engineering to introduce fluorescent proteins is required. The microscopes themselves also have to be maintained by specialists, and require significant data processing.

They are introduced to contrast to the device-based approach of this thesis, which does not require specialists, difficult alignment or sample modifications in order to use.

1.6.1  Structured illumination microscopy

In Structured illumination microscopy (SIM) the input beam intensity is modulated by for example a high frequency grating. The interference patterns between these high input frequencies and high frequency detail beyond the diffraction limit in the image, result in lower spatial frequencies which can be observed[32, 33], see left part of figure 1.6. The grating is rotated in the beam axis to collect interference in all directions. Then an inverse spatial Fourier transform converts interference patterns as a function of rotation angle into additional high frequency detail, compared to conventional far-field microscopy. Resolution obtained in figure 1.6 is of the order of 80 nm.

![Figure 1.6: Left](image) The principle of SIM where interference between periodic excitation (grey) and the image of a grating (black) produce a lower frequency pattern which may be resolved even if the original pattern was beyond the diffraction limit. Rotation and translation of the original grating pattern and computational reconstruction allow this high frequency detail to be recovered. **Right** An example image comparing conventional widefield TIRF microscopy (a–b) to widefield TIRF-SIM (c–d) showing the resolution improvement. The right figure was adapted from [32].
1.6.2 Stimulated emission depletion microscopy

Stimulated emission depletion microscopy (STED) is a point-scanning microscopy where a sub-diffraction limited spot is created by the interaction of two spectrally distinct laser beams. First a Gaussian spot excites fluorescence from chromophores, then second beam with a doughnut shaped focus quenches the fluorescence around the centre of the Gaussian beam, this effectively reduces the incident beam diameter[34–36], see figure 1.7. The obvious improvement in resolution from STED is demonstrated in figure 1.8 with a resolution of 80 nm compared to around 200 nm from the confocal micrograph.

Recent developments have aimed to reduce the short-comings of STED’s design; such
as addressing the alignment difficulty by using single mode fibres[35], and boosting the scanning speed using parallel excitation across the sample in an interferometer design[37].

1.6.3 Photo activated localisation microscopy

![Photo activated localisation microscopy setup](image)

Figure 1.9: An example experimental setup used in PALM or STORM microscopy. It is possible to use a single laser if the chromophore has a long lived inactive form which forms spontaneously upon excitation.

Photo activated localisation microscopy (PALM) is a wide-field super resolution microscopy which aims to track individual chromophores and localise their centres beyond the diffraction limit[38]. Localisation accuracy and fluorescent protein labelling are crucial to this technique[39]. Localisation of a single chromophore position is achieved by exciting a chromophore and monitoring the photons emitted, the localisation precision depends on; the point spread function of the microscope which determines the resolution, the number of photons collected, the background noise and the pixel size of the detector[40, 41]. When sufficient photons have been collected photo-bleaching is initiated by a second laser. The sample is then allowed to relax into the ground state, this avoids over-population of light emitting states. Then excitation is initiated again.

A related technique is stochastic optical reconstruction microscopy (STORM), instead of relying on photo-bleaching to isolate chromophores in time as in PALM, photo-blinking or photo-switchable dyes with a fluorescent and non-fluorescent state are used[42]. Photo-blinking is the transition to a long lived triplet state, before photo-emission.
switchable to states which are non-fluorescent allow modulations in brightness over time such that neighbouring molecules can be measured in isolation and be independently localised[42].

STORM is often used with synthetic chromophores such as the Alexa dyes or other bright cyanine dyes[43], which can be detected to the single molecule level[44]. These chromophores are generally attached to antibodies to target a specific cell structures[43]. PALM is often used with engineered cells which express green fluorescent protein (GFP) in a target structure[45].

GFP is prone to photo-bleaching so using this property as a discriminator in time between molecules is natural, whereas the stability of synthetic chromophores can be significantly higher. Since the discovery of GFP in the Aequorea victoria jellyfish by Osamu Shimomura[46], various mutant GFP strains have been created a selection are detailed[47–50] in table 1.1. The quantum yield, emission and absorption spectrum have all been adjusted, and expression in different cell and animal types is also possible[51].

<table>
<thead>
<tr>
<th>Fluorescent Protein</th>
<th>Excitation λ (nm)</th>
<th>Emission λ (nm)</th>
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<td>[50]</td>
</tr>
<tr>
<td>mEos2</td>
<td>573</td>
<td>584</td>
<td>0.66</td>
<td>46000</td>
<td>323</td>
<td>[50]</td>
</tr>
</tbody>
</table>

a Extinction coefficient.
b The time taken until 50% of initial brightness.

An example image using STORM is shown in figure 1.10 and shows that with STORM the internal structure in the clathrin-coated pits can be observed and the microtubule structure is more clearly resolved with a conservative estimate of resolution of 80 nm or approximately λ/6 [43, 52].
Figure 1.10: A Convention microscopy image of microtubules and clathrin-coated pits, compared to STORM of the same region in B and magnified in C. Excitation was at 457 and 532 nm for the dye pairs Cy2-Alexa 647 and Cy3-Alexa 647, labelling microtubules and clathrin respectively. Figure from [52].
1.7 Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy (FCS) can be used to determine the size of a microscope’s focus and focal volume so is a powerful tool to study nanoscale volumes. FCS is also able to determine the number of molecules present in the focal volume down to the SM level. The technique relies on measuring the rapid changes in fluorescence intensity a function of time and calculating the average time present in the focal volume.

To conduct FCS an excitation laser depicted blue in figure 1.11 reflects off the dichroic, is focused by the objective lens to a diffraction limited spot inside the sample. The sample then emits fluorescence illustrated green, which passes through the objective, dichroic mirror and pinhole into the detection assembly. Where the light is filtered again and then divided by it’s polarisation between two detectors. Each avalanche photodiode detector (APD) is connected to photon counting hardware, which computes the experimental correlation function. For small molecules rotation diffusion occurs on a $10^{-15}$ s timescale, more than an order of magnitude faster than the timing resolution of $10^{-10}$ s. Polarisation splitting in the detection provides an independent measurement of the same event where many hundreds of photons are collected, to cross correlate against.
During the experiment intensity is recorded as a function of time, $\delta F(t)$, which varies about the average $\langle F \rangle$, due to Brownian motion. Combining these terms gives a fluctuation in time, $\delta F(t) = F(t) - \langle F \rangle$. To calculate the average a long time span is required and the autocorrelation function $G(\tau)$, shown in equation 1.7.1, which is a function of the time shift $\tau$,

$$G(\tau) = \frac{\langle \delta F(t) \delta F(t + \tau) \rangle}{\langle F(t)^2 \rangle}.$$  

(1.7.1)

Figure 1.12: Left: The product of the original intensity time data and time shifted replicas after the integration of many shifts and normalisation gives an autocorrelation curve shown Right.

The original signal at a time $t$ is multiplied with time shifted $\tau$ signal replicas these are then integrated and normalised. The notation for integration in this field is denoted by $\langle \rangle$ pair and normalisation is achieved by dividing by the integrated intensity squared, $\langle F(t)^2 \rangle$. The process of autocorrelation calculation may be understood graphically in figure 1.12.

The $G(\tau)$ shown in equation 1.7.1 becomes valid as $t_{\text{meas}} \rightarrow \infty$[53], on the timescale of the fluctuations which at microsecond to millisecond intervals, infinite time can be approximated after recording for approximately 60 to 90 seconds. The signal to noise ratio often dictates that the measurement time is longer however, due to the sensitivity to background signals and the stochastic nature of the measurement.

Single photon avalanche photodiode detectors are prone to after-pulsing, where a second pulse is produced with a 1–3% probability; microseconds after the original single photon is detected[54]. This artefact can seriously disrupt the measurement of the correlation curves at correlation times shorter than 1\mu s seconds[54]. The after-pulse probability varies slowly over a period of hours to days. It has an exponentially decaying profile on the microsecond timescale[54]. Though it is possible to measure the after-pulsing using
light from a non-pulsed light source, the additional noise introduced from measuring this correction reduces the signal-noise ratio. This can preclude measuring signals of SM fluorescence accurately. To reduce the noise in this correction the afterpulsing profile can be collected over a longer time period. The after-pulsing probability shifts over time[54], which can also lower the confidence in the results even though the correlation curve has lower noise.

The most promising solution is to employ two detectors in a cross-correlation scheme[55], here the two detector’s signals are multiplied in time before autocorrelation is calculated[55]. This biases the autocorrelation of the signal against single detector only effects. The most likely event is that the after-pulse’s intensity, is multiplied by the background level of the other detector. This is because it is highly unlikely both detectors will after-pulse simultaneously[54, 55]. In this way fluorescence has a higher weighting since it is detected by both detectors and the signal multiplied and has a larger contribution than after-pulsing to the $G(\tau)$. This is the reason for the dual detection apparatus measuring the intensity fluctuations, as shown schematically in figure 1.11.

### 1.7.1 Data extraction from the correlation function

The correlation function $G(\tau)$ does not directly provide data, instead theoretical models are fit to the correlation function. The fit parameters from these models contain information on the chromophore, inside the confocal volume and also about the confocal volume itself. A popular theoretical model is to assume a single type of chromophore, in a three dimensional focal volume. The model also includes a percentage of chromophores, which are excited into their triplet state $t_{\text{frac}}$, where they cannot absorb or emit light for typically many microseconds. The triplet correction corrects the correlation amplitude, which is a crucial parameter to determine the number of molecules.

Fitting the experimental data provides an estimate for the average time a chromophore spends in the focal volume. The diffusion constant $D$ of a chromophore is the ratio of thermal energy to the ease of moving in a given liquid as given by the Stokes–Einstein equation[56, 57],
CHAPTER 1. INTRODUCTION

\[ D = \frac{k_B T}{6\pi \eta r} \]  

where \( k_B \) is the Boltzmann constant, \( T \) is the temperature, \( \eta \) is the dynamic viscosity and \( r \) is the radius of the particle. For commonly used fluorescent dyes, such as the rhodamine series the value of \( D \) is between \( 3 - 5 \times 10^{-10} m^2 s^{-1} \). Knowledge of the diffusion constant \( D \) and the average diffusion occupancy time in the confocal volume \( \tau_{\text{diff}} \) allow the confocal volume to be calculated. The three dimensional diffusion of a single chromophore with a fraction of the molecules entering the triplet state, is given by equation 1.7.3,

\[ G(\tau) = G_{\text{inf}} + \left( N_0^{-1} \left( 1 + \frac{\tau}{|\tau_{\text{diff}}|} \right)^{-1} C_{\text{conf}} T_{\text{trip}} \right) \]  

where \( G(\tau) \) is the correlation function, \( T_{\text{trip}} \) is the triplet correction term, \( C_{\text{conf}} \) is the confocal correction term these are defined in equations 1.7.4 and 1.7.5. \( G_{\text{inf}} \) is the correlation function at infinity, \( N_0 \) is the reciprocal of the \( G(\tau) \) y-intercept value, \( \tau_{\text{diff}} \) is the half transit time for a species in the measurement volume. \( S \) is the height \( Z_0 \) to width \( \omega_0 \) aspect ratio of the excitation volume written as \( S = \frac{Z_0}{\omega_0} \). \( t_{\text{frac}} \) is the fraction of chromophores in triplet states, \( \tau_{\text{trip}} \) is the triplet state lifetime. The triplet \( T_{\text{trip}} \) and confocal \( C_{\text{conf}} \) correction terms are given by,

\[ T_{\text{trip}} = \left( 1 - |t_{\text{frac}}| + |t_{\text{frac}}| \exp \left( -\frac{\tau}{|\tau_{\text{trip}}|} \right) \right) \]  
\[ C_{\text{conf}} = \left( 1 + \frac{\tau}{S^2 |\tau_{\text{diff}}|} \right)^{-1/2} \]

The confocal volume model can be simplified to account for restricted diffusion for instance in a cell membrane[10] by omission of the \( C_{\text{conf}} \) term in equation 1.7.5. Or if the laser power is low or triplet crossing is not significant the triplet factor \( T_{\text{trip}} \) can be omitted from the fitting model in equation 1.7.5. Such simplifications reduce the number of degrees of freedom in the model, and increase the confidence in the fitted parameters. This is valuable where the data has significant noise, or a term is not representing the data well.
The number of molecules $N$ is calculated by the product of spatial intensity distribution $\gamma$, and the fitting model term $N_0$,

$$N = N_0 \gamma$$  \hspace{1cm} (1.7.6)

where in confocal geometry $\gamma = \frac{1}{4\gamma_0} = 0.3535^{[59]}$. The lateral focal size $\omega_0$ is calculated from the half average diffusion time $\tau_{\text{diff}}$ and the diffusion constant $D$ in equation 1.7.7.

The effective confocal volume $V_{\text{eff}}$ is calculated by equation 1.7.8,

$$\omega_0^2 = 4D\tau_{\text{diff}}$$  \hspace{1cm} (1.7.7)

$$V_{\text{eff}} = \pi^{3/2}S\omega_0^3.$$  \hspace{1cm} (1.7.8)

An example of using a function fitting procedure to extract molecular level detail is given below in table 1.2

Table 1.2: Example data extracted from a FCS experiment using a rhodamine 110, with a known diffusion coefficient $D = 4.3 \times 10^{-10}$ m$^2$s$^{-1}$[58] and extracting the size of the confocal volume, measured in fL ($1 \times 10^{-15}$L).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Extracted quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{\text{int}}$</td>
<td>$4.46 \times 10^{-4}$</td>
<td>NA</td>
</tr>
<tr>
<td>$t_{\text{fasc}}$</td>
<td>$1.04 \times 10^{-1}$</td>
<td>10.4 % triplet crossing or bleaching</td>
</tr>
<tr>
<td>$\tau_{\text{trip}}$</td>
<td>$2.29 \times 10^{-3}$</td>
<td>2.29 $\times 10^{-3}$ ms triplet lifetime</td>
</tr>
<tr>
<td>$N_0$</td>
<td>$8.81 \times 10^{-1}$</td>
<td>2.5 molecules</td>
</tr>
<tr>
<td>$\tau_{\text{diff}}$</td>
<td>$1.39 \times 10^{-4}$</td>
<td>139 $\mu$s half diffusion time</td>
</tr>
<tr>
<td>$S$</td>
<td>3.79</td>
<td>$Z_0/\omega_0 = 3.79$</td>
</tr>
<tr>
<td>$Z_0$</td>
<td>$1.85 \times 10^{-6}$</td>
<td>1850 nm axial confocal size</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>$4.89 \times 10^{-7}$</td>
<td>489 nm lateral confocal size.</td>
</tr>
<tr>
<td>$V_{\text{eff}}$</td>
<td>$2.47 \times 10^{-15}$</td>
<td>2.47 fL confocal volume.</td>
</tr>
</tbody>
</table>

Time correlated single photon counting (TCSPC) is a steady state technique to study fluorescence excited state dynamics and is detailed in the next section.
1.8  Time correlated single photon counting

1.8.1  Background theory

The principle of TCSPC is to measure the time it takes a molecule to emit a photon of light, after the excitation pulse is incident on the sample[60], the fluorescence lifetime (FL). This is done by measuring a sample’s fluorescence in a series of time channels, if a photon arrives in a given channel the total number of photons counted with that channel is incremented. These measurements are brought together into a histogram with the number of photons on the y-axis and time on the x-axis[60]. The form of this distribution is chromophore dependent, the simplest case is single exponential decay often occurring for small molecules as in figure 1.13. Multiple exponential decay is also possible[61] with more than one strong non-radiative decay mechanism is present, and they occur at different rates, this is illustrated in figure 1.14.

Figure 1.13: Semi-log graph of a series of idealised mono-exponential decays with varying FL in the range 1–8 ns showing their linear trend on the logarithmic axis.
1.8. TIME CORRELATED SINGLE PHOTON COUNTING

Figure 1.14: Semi-log graph of idealised bi-exponential decays with a variable second component FL from 3–24 ns compared to a mono-exponential decay. A non-zero second exponential adds curvature to the trend.

The form of the mono-exponential curve is given in equation (1.8.1)[20] and the multi-exponential form is given in equation (1.8.2)[20],

$$I_{t, \text{single}} = I_0 \exp \left( -\frac{t}{\tau} \right)$$ (1.8.1)

$$I_{t, \text{multiple}} = \sum_{i=1}^{n} I_{0,i} \exp \left( -\frac{t}{\tau_i} \right)$$ (1.8.2)

where $I_0$ and $I_t$ denote initial intensity and intensity at the time $t$, respectively, and the FL $\tau$. This is the $1/e$ time in the exponential decay of the initial maximum, and also the average time for fluorescence emission. $I_0$ is the pre-exponential and sets the initial intensity in the decay profile and $\tau$ dictates the rate of decay of the profile.

1.8.2 Fluorescence lifetime estimation

Since the excitation pulse-width is non-zero, in experimental results the top of the decay is often distorted especially with short decay times, this is depicted in figure 1.15. This distortion arises when fluorescence photons arrive at the detector while the excitation pulse is still active on the sample. In a semi-log plot the decay curve can be fitted to extract the FL.
CHAPTER 1. INTRODUCTION

Figure 1.15: Normalised decay profile of Rhodamine B at 21°C in ethanol. The instrument response function full width at half maximum is 305 ps. Insert shows the molecular structure of Rhodamine B.

If the short-time information is desired it may be estimated by computational re-convolution[62, 63]. Re-convolution is a time-domain iterative fitting technique, where the instrument response is multiplied at all points in time with a model decay function, which is adjusted until it best represents the data. This is not to be confused with convolution is the multiplication of the Fourier coefficients of two functions, followed by the inverse Fourier transform[64].

To measure the laser pulse profile, the laser is scattered from a non-fluorescent colloidal solution, this is termed the instrument response function (IRF) shown blue in figure 1.15. The IRF is often Gaussian in profile with small after-pulses or a low intensity decay from the signal peak as is present in figure 1.15. For the simplest case of a mono-exponential decay, only the FL $\tau$, curve height $I_0$, background intensity offset and the position in time the decay occurs in time are optimised. Least squares regression is a popular curve fitting method. After an initial guess of parameters the fitting error is determined for all points being fit and squared, summed and is minimised in further iterations, to minimise the error between the data and the fit[65, 66].

The fit quality is determined using the standard reduced chi squared $\chi^2_{\text{red}}$ measure, the best quality fits are generally $\chi^2_{\text{red}} \rightarrow 1$ in the empirically determined range $0.8 \leq \chi^2 \leq 1.2$. With $\chi^2_{\text{red}} < 1$ indicating an "over-fit" to the data and $\chi^2_{\text{red}} > 1$ indicating a "poor fit"[67].

52
For more complicated decay models or data containing other contributions the fit may not be as ideal and the confidence in the fit decreases. The equation for the reduced chi squared $\chi^2_{\text{red}}$ has the form,

$$\chi^2_{\text{red}} = \frac{1}{df} \times \sum \left( \frac{(f_{\text{obs}} - f_{\text{exp}})^2}{\sigma^2} \right)$$  \hspace{1cm} (1.8.3)

where $df$ is the number of degrees of freedom, $f_{\text{obs}}$ are the experimentally observed data points, $f_{\text{exp}}$ are the expected modelled results of the fitting and $\sigma$ is the variance.

The error at each point is well defined, as the square root of the intensity gives the standard deviation of the measurement. This is because photon counting follows Poisson statistics[68].

### 1.8.3 TCSPC experimental details

![Figure 1.16](image)

Figure 1.16: A schematic showing the experimental design of TCSPC, with inserts showing the advantages of using a constant fraction discriminator to reduce signal jitter, and the voltage-time ramp of the time-to-amplitude converter.

The TCSPC setup is shown in figure 1.16. The constant fraction discriminator generates a trigger giving a uniform triggering time, for signals of variable intensity. This avoids the "time walk" seen with intensity threshold triggering[69], this is illustrated in figure 1.16 insert A.

The laser pulse triggers a photodiode, the signal then passes through a constant fraction
discriminator to generate a trigger pulse. Then the trigger is passed to the time-to-amplitude converter[20], where the voltage ramp shown in insert B is started, by analogy to charging a capacitor. The time-to-amplitude converter is stopped once the fluorescence photodetector has detected a fluorescence signal, the signal from which is also routed through a constant fraction discriminator. The voltages are converted into their corresponding times by calibration of the voltage ramp versus time, ideally a linear system response is desired for uniform accuracy. This signal voltage is then digitised by an analogue to digital converter, and sent to a PC where it forms a histogram of photon arrival times.

1.8.3.1 TCSPC Experimental Considerations

It is important to ensure the total absorption of the sample is measured to be lower than 0.1 to avoid inner filter effects, which cause apparent decreases in FL because the sample is reabsorbing some of the emitted fluorescence[70]. Serious errors with the optical alignment can cause stray reflections, which can cause short time-scale anomalies. These however are clearly visible when calibrating the system by observing the decay profile of a known standard.

1.8.4 Significance of fluorescence lifetime

Comparisons of FLs can be used to report on changes in the chromophore environment, ranging from changes in temperature, electronic energy levels, distance between two chromophores in fluorescence resonance energy transfer[71]. FLs are also affected by chromophore proximity to metal nanostructures, chromophores transfer their excitation energy to the metal’s surface electrons reducing the FL, see section 1.9.

Given that there is sufficient differentiation between the absorption and emission spectra, multiple chromophores may be probed in the same environment.

Comparisons between different samples do not require knowledge of the individual component concentrations, the results are not affected by elastic (Rayleigh) scattering as this occurs at a much shorter time-scale[72] and is clearly distinguishable. In TCSPC applications chromatic aberrations or other consistent optical imperfections also do not effect the FLs providing the fluorescence is received.
1.8.5 Fluorescence anisotropy

When rotational diffusion is slow due to solvent crowding or a large analyte there may be differences in emission for different polarisations of light[20]. Excitation occurs most efficiently when aligned parallel to the chromophore transition dipole moment. Therefore for slowly rotating chromophores the fluorescence polarisation parallel to this dipole moment will have a greater photon flux, until rotation occurs. The greatest fluorescence emission occurs at short time, close to the excitation pulse. The fluorescence polarisation anisotropy $r$ is defined by equation 1.8.4[20],

$$r = \frac{GF_\parallel - F_\perp}{(1 - 3l_2)GF_\parallel + (2 - 3l_1)GF_\perp}$$ (1.8.4)

where $G$ is ratio of parallel and perpendicular detection efficiencies, $F_\parallel, F_\perp$ are the signal intensities of the parallel and perpendicular polarisation resolved fluorescence detection channels. $l_1$ and $l_2$ are calibrated polarisation contamination factors as a result of the microscope objective lens not preserving polarisation perfectly. The polarisation anisotropy $r$ is also related the FL $\tau_{\text{decay}}$ by equation 1.8.5[20],

$$r = \frac{r_0}{1 + \frac{\tau_{\text{decay}}}{\rho_{\text{diff}}}}$$ (1.8.5)

where $r_0$ is the fundamental anisotropy a constant for a given homogeneous solvent-chromophore combination. $\rho_{\text{diff}}$ is the rotational correlation time of the chromophore, after which the fluorescence polarisation is randomly orientated.
CHAPTER 1. INTRODUCTION

1.9 Surface plasmons

1.9.1 Introduction

Negative refractive index metamaterials often contain metals when operating in the visible region of the spectrum so an elementary understanding of metal electronic structure will help further discussions.

1.9.2 Drude model for electron motion in metals

The Drude model is a simple model of the electronic structure of non-magnetic metals inside an applied electric or electromagnetic field $E$[73, 74]. The forces on electrons from the external electric field are equal to $eE$.

The internal dampening force can be expressed as $m_e \frac{-v}{\tau}$, where $v$ is the velocity, $m_e$ is the electron mass, and $\tau$ is the average time between collisions. So $1/\tau$ is the collision frequency, the value of $\tau$ is metal dependent, for silver it is 0.195 picoseconds[75]. The equations of motion for a surface electron in the Drude model is given by the following equation,

$$m_e \frac{dv}{dt} + m_e \frac{-v}{\tau} = -eE = F$$  \hspace{1cm} (1.9.1)

where $m_e$ and $e$ are the electron mass and charge, $v$ is the velocity and $E$ is the applied external field. One of the most useful results of the Drude model is the effective dielectric permittivity, $\epsilon_{\text{eff}}$ given by the following expression,

$$\epsilon_{\text{eff}} = \epsilon_0 \left(1 - \frac{\omega_p^2(1 - i/\omega\tau)}{(1/\tau^2 + \omega^2)}\right)$$  \hspace{1cm} (1.9.2)

where $\epsilon_0$ is the free space permittivity, $i$ is an imaginary number and denotes an absorption term in the permittivity. $\omega$ is the angular frequency of the incident light and $\omega_p$ is the plasma resonance angular frequency, at which the electron plasma is anti-resonant. This is given by equation 1.9.3,
1.9. **SURFACE PLASMONS**

\[
\omega_p^2 = \frac{N e^2}{m_e \epsilon_0} \tag{1.9.3}
\]

where the electron density of the metal \( N \), determines how many free electrons are able to oscillate. On the surface of silver for instance there are \( N = 5.85 \times 10^{28} \text{ m}^{-3} \). The plasma frequency \( \omega_p \) is central to the understanding of light-metal interactions, and is located at 138 nm according to the Drude model for silver metal. Experimentally plasma frequencies are geometry dependent and sensitive to the electrical response of their environment. Plasma resonance wavelengths range between 400–580 nm in silver\[76, 77\], in reasonable agreement with the Drude model given the simplicity of the theory. Deviations from the Drude model are due to geometry factors not considered in the theory, which drastically effect the resonance frequency[76–80].

The absorbance maxima is at \( \omega_p \) where absorption causes the electrons to form an oscillating dipole on the surface of the metal. The magnitude of this is metal and geometry dependent, the geometry changes the metal’s surface area and therefore the metal’s resistance at high frequencies.

This oscillating surface of electrons has a strong electric and magnetic field associated with it. In metals the polarisable electrons are confined to the surface of the metal, this naturally localises the excitation energy of the incident light source to the surface, these are called surface plasmons (SP).

In order to excite SPs there needs to be a scattering event, since there is a mismatch in the spatial angular frequency otherwise known as momentum \( k \), \( k = 2\pi / \lambda \) between the photon and electron, as shown by,

\[
k_{\text{light}} = \frac{\omega}{c} \tag{1.9.4}
\]

\[
k_{\text{SP}} = \frac{\omega}{c} \left( \frac{\epsilon_1 \epsilon_2}{\epsilon_1 + \epsilon_2} \right)^{\frac{1}{2}} \tag{1.9.5}
\]

where \( \omega \) is the angular frequency of the light and the plasmon, \( c \) is the speed of light. \( \epsilon_1 \) and \( \epsilon_2 \) are the permittivity values of material 1 and 2 respectively. \( k_{\text{light}} \) and \( k_{\text{SP}} \) are the
spatial angular frequencies of the light and surface plasmon respectively, describing the number of oscillation periods per unit length.

The Drude model predicts a $k_{SP}$ for a 450 nm wavelength surface plasmon, at the silver-glass interface, $k_{SP} = 1.53 \times 10^7$ m$^{-1}$. This is approximately 10% larger than the that of the incident 450 nm light, $k_{light} = 1.40 \times 10^7$ m$^{-1}$, both light and SPs share a common $\omega$ value.

### 1.9.3 Surface plasmon coupling

Coupling light into SPs can be achieved using metal gratings[81] or evanescent field coupling from total internal reflection close to a metal surface.[82][83], see figure 1.17.

Figure 1.17: Schematic of the Otto[82] and Kretschmann configurations[83] for exciting SPs, on the left and right respectively. The blue region schematically shows the evanescent field intensity from the total internal reflection.

Coupling the relatively small wavevector of light $k_{light}$ into the larger SP wavevector $k_{SP}$, is achieved using the high spatial frequency waves produced by evanescent fields. Total internal reflection is one method to produce an evanescent field at the interface, as was discussed in section 1.5.1. The Kretschmann configuration[83], requires an optically thin layer of a plasmonic metal to be deposited on the prism, inside which, the total internal reflection occurs. The Otto configuration[82] by contrast, can operate on a bulk metal, but requires careful positioning close to the surface. Coupling into a SP is observed as a trough in the surface reflectivity as a function of angle, implying the absorption by a SP mode. The optimal coupling angle $\theta_{ex}$ is achieved when the wavevector of incident light $k_{light}$ is described by,

$$ k_{light} = \frac{\omega}{c} \sqrt{\epsilon_{prism}} \cdot sin\theta_{ex} $$
where $k_{\text{light}}$ is the wavevector of the incident light, $\theta_{\text{ex}}$ is the incident angle of the light, $\omega$ is the angular frequency of SPs and light and $\epsilon_{\text{prism}}$ is the relative permittivity of the prism.

The type of SP mode excited propagates across the surface radially from the exciting evanescent field. This makes this technique suitable for wide-field microscopy and it is relatively easily deployed. The position of the absorbance peak is used in sensors to detect variations in local permittivity[84]. This technique has been used to monitor the growth of monolayers and to detect binding of biological molecules to surface attached antibodies[85].

### 1.9.4 Plasmon losses

Losses in SPs are mainly divided into two categories; radiative and non-radiative, where non-radiative are due to absorption by the metal, and radiative are caused by scattering events on the metal surface due to surface roughness. To reduce loss the metal crystal structure may be improved by annealing[86] allowing greater propagation lengths. Radiative losses may be reduced by optimising the surface roughness[81] to avoid unwanted scattering events which couple SPs into light[87].

In order to describe the absorption inside a metal, the relative permittivity is modified by adding an imaginary term $\epsilon_{\text{imag}}$. The real term represents the energy loss in a material as a response to the incident light.

\[
\epsilon_{\text{complex}} = \epsilon_{\text{real}} + i\epsilon_{\text{imag}} \tag{1.9.7}
\]

These losses are a function of both the type of metal and the wavelength of incident light as shown in figure 1.18. The type of metal used depends on the wavelength of interest; gold is optimal above 600 nm and silver is used above 400 nm.
1.9.5 Local surface plasmons

An important class of SP termed a local SP can be coupled locally around scattering nanostructures smaller than the incident light’s wavelength. The local SP is confined to the surface of a nanoscopic object so the resultant evanescent fields are much stronger than the incident light which produced them. This leads to the term SP enhancement or quality factor $Q$ defined as,

$$Q = \frac{\text{Local electric field}}{\text{Incident electric field}}$$  \hspace{1cm} (1.9.8)

where the quality factor $Q$ defines the relative electric field strength around a local SP and can have factors as large as $10^8$[89]. Quality factors $Q$ for SPs are a function of geometry[90] for local SPs, and are given by equations 1.9.9 and 1.9.10 for spherical and rod geometry respectively,
where \( Q_{\text{sphere}}(\omega) \) and \( Q_{\text{rod}}(\omega) \) are the quality factors, of a sphere and rod, as a function of the light’s angular frequency. \( \epsilon_{\text{real}}(\omega) \) and \( \epsilon_{\text{imag}}(\omega) \) are the real and imaginary parts of the permittivity of the metal as a function of the light’s angular frequency. The real part correspond to the amplitude of the material response, and the imaginary part corresponds to the material absorption loss. These relations are illustrated graphically in figure 1.19.

In gold and silver the sharpest relative decrease in the \( Q \) factor occurs close to the metal’s plasmon resonance, at around 450 nm and 600 nm for silver and gold respectively. Close to and below the plasmon resonance wavelength, internal absorption or resistive loss becomes significant. For gold and silver nanoparticles this plasmon resonance is observed as peak in absorbance. This varies with nanoparticle size, where larger particles have a longer wavelength resonance. This relationship is shown in figure 1.20, the peak width varies with the particle size distribution and the inter-particle spacing. With larger
distributions having contributions from red and blue shifted resonances, and more closely spaced particles having greater displacement current induced in their neighbours due to the intense oscillating electric field[78].

Figure 1.20: Absorption spectra of silver and gold spherical nanoparticles as a function of size. The absorption peak corresponds to the SP resonance. For silver the SP resonance varies from 420 nm to 460 nm for 3 nm and 20 nm particles respectively. For gold the SP resonance varies from 520 nm to 575 nm for 9 nm and 99 nm particles respectively. Figures adapted from [91, 92].

The required structures can be engineered using lithography or chemically synthesised solution nanoparticles, which can adopt a large variety of shapes. These shapes vary according to the conditions used in their synthesis[93].
1.10 Research context

Development of nanoscale focusing volumes as an alternative to the far field super resolution approaches has promise as a more cost accessible solution for super resolution imaging. The benefits are that it can: utilise existing microscopes, avoid complicated laser alignment, needs no post processing, avoids the need for multiple image captures and would allow ultra-fast nanoscale imaging. The imaging timescales could be as short as the picosecond and nanosecond level so FL resolved super resolution could be possible. It is known that biologically important protein dynamics occur on a picosecond to nanosecond timescale[94], and that this enables larger protein motions to occur. Allowing ultrafast dynamics to be mapped at improved spatial resolution would be a clear advance.

Small focusing volumes are key to allowing high concentration single molecule studies. For instance in enzyme kinetics or for spatially resolving features in a live cell, these require strong excitation isolation. Metal films with apertures inserted provide a way to achieve this small volume. This is achieved by blocking all-but a small portion of the incident light, which is concentrated close to the bottom of the aperture.

It has been predicted that a near-field lens such as a negative refractive index medium[15] can help to create a smaller focusing volume. Metal nanowire (NW) arrays appear very promising and also have the advantage of plasmonic enhancement of emission rates[29, 95–98] and directionality of emission along the SP resonant axis[99]. Different metal NW feature-size regimes will be explored, from the strongly sub-wavelength $\lambda/22$ to the often ignored $\lambda/5$, where features interact more strongly and are less coupled to their neighbour due to increased spacing.
1.11 Thesis outline

The second chapter details the fabrication of nanopores in aluminium films with electron beam lithography, with results illustrated by microscopy and results from the application to FCS experiments. In the third and subsequent chapters, a materials approach to light confinement was adopted because of the loss of the lithography equipment. The third chapter deals with the structural templates around which the new material are constructed using the anodising technique, producing the hexagonal porous alumina template structures. In the fourth chapter different methods of filling the alumina templates with metal are trialled, to produce NW arrays on a glass substrate. In the fifth chapter, optical characterisation of the vertical metal NWs on glass is undertaken: to examine their focusing properties, absorption spectra, near-field FL suppression properties. In the sixth chapter there is a summary of the work as a whole and possible directions for future work are evaluated.


CHAPTER TWO

ZERO MODE WAVEGUIDES
2.1 Introduction

Single molecule studies require the isolation of chromophores. In traditional microscopy, optical isolation has been achieved by using very low chromophore concentrations, typically in the picomolar range. This motivated the development of metal nanoapertures, which were referred to as "zero mode waveguides" (ZMW) to increase the optical isolation and allow SM studies at higher concentration. A schematic of the original experimental arrangement is shown in figure 2.1, which depicts an inverted confocal microscope with excitation focused onto a ZMW pore, at the base of the ZMW pore an enzyme was surface attached. The original aim was to allow single molecule enzymology studies with micromolar concentrations of a fluorescent reactant in the solution above the ZMW. Micromolar concentrations are closer to those found in biological systems, so may help provide realistic reaction rates.

Figure 2.1: Schematic of confocal microscope with a ZMW device on a fused silica substrate. At the base of the pores an enzyme was attached and the reaction was monitored by measuring the fluorescence residence time of the labelled substrate. Figure adapted from [1].

Using FCS the residence times inside the ZMW confocal volume were measured were greatly reduced from $30 \times 10^{-6}$ seconds to $2.5 \times 10^{-6}$ seconds, a reduction by a factor of 12[1]. This decrease in residence time allowed the concentration to be increased by a factor
of 2500 and obtain the same correlation amplitude as the control, the concentrations were 10000 nM and 4 nM respectively. Note that correlation amplitude is inversely proportional to the number of chromophores, see section 1.7 for more details. In the experiment they used rhodamine 110 fluorescent labels on the enzyme substrate to monitor the reaction rate via the residence time in the ZMW pore[1].

Figure 2.2: A Unnormalised and B normalised FCS curves of the diffraction limited control compare to ZMW showing the large increase in concentration possible with ZMW because of the greatly reduced focal volume. Figure adapted from [1].

ZMWs spatially select only a portion of the excitation light, this light then enters the base of the ZMW and is attenuated axially, this reduces the excitation volume vertically and horizontally. An additional benefit of the ZMW configuration, is the excitation of local SP modes at the base of the ZMW, at the glass-metal interface. The electric field produced from the SP is evanescent so is also short range and does not extend significantly into the concentrated solution above the ZMW. The resulting excitation volume is up to 3–6 orders of magnitude smaller than conventional confocal microscopy[1–3]. The ZMW are produced periodically with a period of several microns, to ensure that the evanescent fields of neighbouring ZMW do not significantly overlap, to prevent unwanted transmission[4].

The local SPs excited in ZMW nanoapertures lead to enhancements in molecular brightness, which lead to improvements in the signal-to-noise ratios of fluorescence studies sensitive on the SM level[5]. Enhancements of between 6.5 to 25[6, 7] have been reported in the literature. The enhancement depends on the specific dye employed, the metal used in the ZMW and the laser intensity, as well as surprising aspects such as the adhesion layer used[7]. The brightness or count-rate, per molecule for aluminium and gold is compared in figure 2.3, where gold appears to be more efficient. However, the comparison is conducted
at 633 nm close to the SP resonance for gold[8, 9].

![Graph showing the comparison of the count-rate per molecule of Alexa-Fluor 647 excited at 633 nm with a He-Ne laser, adapted from [10].](image)

Figure 2.3: Comparison of the count-rate per molecule of Alexa-Fluor 647 excited at 633 nm with a He-Ne laser, adapted from [10].

There are disadvantages to ZMW however, they require high precision patterning to create features which are 70–350 nm in diameter. The cost of this patterning is quite prohibitive to widespread adoption. In addition to patterning the metal layer deposition also requires expensive, low throughput vacuum deposition techniques. These factors restrict the ZMW devices to a research proof of concept, but further developments are needed to apply this work in a more cost effective manner. Studies have identified is a long-lived surface adsorbed states[11] and high background signal from reflection and transmission through the ZMW[12].

### 2.2 Aims and objectives

The aim of this chapter was to manufacture, characterise and test ZMW devices on a microscope cover slip substrate. The purpose of this work is to provide a drop-in improvement of optical confinement for SM FCS.
2.3 Experimental

2.3.1 Electron Beam Lithography

The manufacture of zero mode waveguide devices is challenging since they are nanoscopic, with dimensions between 60–300 nm and heights of around 100–150 nm[1–3]. One of the most popular manufacturing techniques using electron beam lithography is outlined next.

2.3.1.1 Scanning Electron Microscope

A scanning electron microscope (SEM) is an evacuated system capable of producing a focused beam of high energy electrons, with a resolution of the order of 10 nm.

The experimental apparatus is shown schematically in figure 2.4[13]. Inside the SEM high vacuum chamber the electron source is a tungsten or LaB$_6$ electron gun, which is heated to 500–2500 K, where it begins to emit electrons via thermionic emission. Electrons are then accelerated towards the positive anode and into the first spray aperture, which acts as a pinhole. The electrons are then condensed by a magnetic lens and passed through further apertures, and refocused by the magnetic objective lens onto the sample. The beam is scanned across the sample using scan coils inside the final condenser lens, which deflect the beam electrostatically in a raster pattern.

This electron beam can also be used for lithographic purposes, by writing into an electron sensitive media termed an electron beam resist, which are detailed in section 2.3.2. A specialised exposure design package on the SEM control computer is used to control the SEM writing process. This package is termed a pattern generator. The pattern generator controls the exposure pattern and the exposure dose.
The exposure dose is critical to the design of an exposure, and governs the amount of electrons incident on the sample per unit area. The dose for single points and areas is quantified by equations 2.3.1 and 2.3.2, respectively, and are shown below,

\[
Dose_{\text{Single Point}} = BeamCurrent \times DwellTime
\]  \hspace{1cm} (2.3.1)

\[
Dose_{\text{Area}} = \frac{BeamCurrent \times DwellTime}{StepSize^2}
\]  \hspace{1cm} (2.3.2)

where the \textit{dwell time} is the time the electron beam exposes a point on the resist and the \textit{step size} is the distance between each exposure point[14, 15] within the area.

### 2.3.2 Electron Beam Resists

Electron beam lithography is the process of writing structures into an electron sensitive material known as a resist, which forms a binary mask. This mask can then be used to define metal features see section 2.3.6, or as an etch mask to pattern an existing layer see section 2.3.5. The processes which occur upon electron exposure are detailed next.
2.3. EXPERIMENTAL

2.3.2.1 Electron Beam Resist Tone

Electron beam resists exhibit two possible responses to electrons, either becoming more or, less soluble in a developer solution. Positive tone resists undergo chain breaking and degradation processes, which increase the solubility of the exposed regions. Negative tone resists become less soluble due to chemical or physical cross-linking processes. So a single point exposure in a positive tone resist would give a pore and the same exposure in a negative tone resist would give a pillar, upon development in developer solution.

2.3.2.2 Electron Scattering Events On Exposure

As the electron beam propagates into the underlying substrate, collisions with atoms and molecules can generate secondary electrons at large scattering angles up to $80^\circ$[14, 15]. The range of these secondary electrons however, is short due to their relatively low energy compared to the incident electron beam. Forward and backward scattering are the two types of electron scattering, forward scattering occurs in resist and backwards scattering is from the underlying substrate.

Forward scattered electrons are caused by inelastic scattering, primarily from the resist electrons, the effect is decreased with increasing acceleration voltages. Forward scattering occurs only at small angles and results in only relatively small feature broadening, vertically down the resist thickness profile[14, 15].

Backwards scattering occurs at large angles and is caused by direct electron-nuclei collisions. Backscattering occurs when electrons collide with substrates containing relatively heavy elements, and the collisions occur elastically[14, 15]. The number of back scattered electrons can be decreased by increasing acceleration voltage, as the electrons interact less strongly with the substrate at higher energies[14, 15].

Another effect which can cause significant feature broadening in resist is the proximity effect, which is shown in figure 2.5[14, 15]. This occurs when points of electron exposure are close together and can result in overexposure and feature enlargement. There are empirical corrections to reduce the dose to compensate[16], but in dense, complex patterns it requires multiple parametrisations to find the correct dose.
Figure 2.5: Showing the electron scattering events upon electron beam exposure. It also illustrates how exposure proximity can cause over-exposure in B, with the "proximity effect". The electrons shown red and black are the large angle, back scattered electrons; and the small angle forward scattered electrons.

A common type of dual-tone resist used for high resolution electron beam lithography is poly(methyl methacrylate) (PMMA), as shown in figure 2.6 and the chemical mechanism showing how it can act as a positive resist is shown in figure 2.7[13]. Features as small as 10 nm have been produced using polymethyl methacrylate as both a positive[17] and negative tone resist[18].

Figure 2.6: Shows methyl methacrylate and poly methyl methacrylate after polymerisation.

Figure 2.7: Shows the free radical chain scission reaction mechanism, by which polymethyl methacrylate acts as a positive resist.
2.3.3 Spin coating

Spin coating is a method of creating thin films across planar substrates, with good uniformity over wide areas. It is used widely in the semiconductor industries to apply resist coatings to substrates ready for patterning. The resist to be coated is dissolved in a volatile solvent and is spun at high speed, spreading the initial droplet radially, reducing the thickness as this occurs. The key parameters controlling the final thickness coating are the spinning speed, mixture viscosity, evaporation rate and acceleration rate[19].

Figure 2.8: Shows the spin coating process, where a droplet is spun at high speed and coats the substrate as a thin film. Excess is shed from the sides and residual casting solvent evaporates rapidly from the thinned film.

2.3.4 Physical vapour deposition

To coat metal onto a substrate surface, the sample is placed 15–20 cm above an evaporating crucible inside a high vacuum chamber, which is pumped to less than $1 \times 10^{-6}$ mbar. The pressure is lowered to this extent to reduce the probability of contamination from oxygen and water inside the chamber especially, and to produce a more uniform result. A high current is then shorted to ground across the crucible to generate required heat to evaporate the coating material.

Sputtering is the physical atomisation of a material using a noble gas plasma and subsequent condensation of the material on the sample. The plasma requires an impedance matching network and careful calibration of the parameters such as noble gas flow rate, gas pressure, sample position, power input into the plasma, coating and cooling times.

The film thickness is monitored during deposition by quartz crystal monitor. The frequency response of the crystal indicates the thickness of the coating, provided the material density, acoustic dampening of the material and original crystal frequency are known.
CHAPTER 2. ZERO MODE WAVEGUIDES

2.3.5 Chemical Etching

Chemical etching is a method of patterning a nanoscale layer where an etchant attacks and dissolves exposed areas, while retaining areas masked often using a resist based etch mask. In metal etching the stages of etching are oxide removal and then subsequent attack of the metal to generate ions and then stabilising those ions in solution.

Zero mode waveguides are predominately made using aluminium[1], for which a common etching composition is a mixture of: H$_3$PO$_4$, HNO$_3$, CH$_3$COOH and H$_2$O. These are mixed in the proportions of 73 wt% : 3.1 wt% : 3.3 wt% : 20.6 wt% respectively, adapted from[20]. The phosphoric acid H$_3$PO$_4$, attacks the oxide layer, while nitric acid HNO$_3$, attacks the exposed aluminium metal. Water H$_2$O and acetic acid CH$_3$COOH help to buffer against changes in pH, since the produced aluminium oxide is amphoteric.

The main disadvantage of chemical etching is that it undercuts the etch mask at the same rate that it etches the desired material, this is termed isotropic etching. So etching through a 50 nm area on a 50 nm thick metal film, would produce a 100 nm diameter spherical hole in the aluminium film[21]. The processing steps in chemical etching and the final etch profile are shown below in figure 2.10.
2.3. EXPERIMENTAL

Figure 2.10: Schematic cross-section of the etching process. A top resist is layer exposed, B exposed resist selectively removed during development, C bottom metal layer etched. The final step (not shown) would be removal of the top layer of the resist.

2.3.6 Lift-off Processing

Electron beam lithography and lift-off processing can be used to generate nanometer scale structures in metal by writing into an electron sensitive polymer known as a resist. Subsequent to writing, undesired portions of the resist are washed away during development to generate a template. Metal is deposited into the template during metallisation, which is then removed along with excess metal during lift-off as detailed below in figure 2.11. Metal deposition is achieved using high vacuum evaporation or sputtering processes which are described in detail in section 2.3.4.

Figure 2.11: Lift-off processing, electron exposure creates differences in solubility, during development more soluble regions are removed. After metal deposition the exposed edge of resist is attacked, and then resist lifted off with the unwanted metal removed too leaving just the desired metal features.
2.4 Zero Mode Waveguide Manufacture

The manufacture methods are summarised in figure 2.11 and 2.10. Cleaning the substrate was done in four stages, first it was washed twice with acetone followed by ultra-sonication for five minutes after each wash, with a final wash with iso-propyl alcohol. The PMMA electron beam resist was then spin coated onto the substrate. It was spun for a total of 60 seconds at 4000 rpm with a 10 second acceleration and deceleration time, giving a thickness of 300 nm. After which it was soft baked at 180°C for two minutes to remove the remaining volatile anisole casting solvent. The resist was then exposed in a 200 μm area using a tungsten filament of a converted Cambridge Instruments Zeiss S360 SEM, controlled by the Elphy Quantum pattern generator.

After electron beam exposure, the resist was developed for 30 seconds in a 1:3 mixture of methyl iso-butyl ketone and iso-propanol, followed by a 15 second wash in iso-propanol. To make the ZMW via lift-off the exposure was tuned high enough to turn PMMA into a negative tone resist and create isolated pillars. Aluminium was then evaporated onto the pillars created before this to a thickness of 50 nm, as determined by Edwards FTM6 film deposition thickness meter, this is detailed in section 2.3.4. 50 nm was chosen since the resist was applied 300 nm thick and this left a 250 nm high resist region exposed. Lift-off of the unwanted material was achieved by soaking and mild agitation using a pipette in acetone, which attacked the exposed polymer, while the agitation helped to remove the thin metal film on top.

To make the ZMW via chemical etching the aluminium was first coated onto a glass substrate then a layer of resist spun on top as detailed above, exposure at low dose where PMMA was a positive tone resist gave holes in the resist. These holes were then used as a binary mask to etch the underlying aluminium as detailed in section 2.3.5 the etch time was varied between 1.5–6.5 minutes.
2.4. Electron exposure details

The beam voltage was set to 25 KV and allowed to stabilise for 90 minutes prior to writing as the tungsten emission source heated up, ensuring a stable writing current. The lithography system’s distance measurements were calibrated by alignment to a characterised micron-scale chessboard pattern. This corrected for any variation in alignment, which had occurred over time or between changes in emission filament. The system was then ready to expose the resist, so the SEM was focused on previously made score marks on the sample surface and then translated to an adjacent open area and exposure control was transferred to the pattern generator.

2.4.1.1 TCSPC equipment used

The laser used was a proprietary Fianium SC400 super-continuum pulsed fibre laser with a 6 ps fundamental pulse width, generating a 300 ps broadband pulse. The broadband spectrum or "super-continuum" is created by many repeated non-linear processes occurring in a photonic crystal fibre[22, 23]. The broad nature of the laser spectrum is illustrated in figure 2.12 left, and after filtering by selective reflection and glass filters right giving a laser peak centred at 470 nm.

![Figure 2.12](image)

**Figure 2.12:** Left: Original spectrum of the commercial Fianium SC400 laser at 10 MHz, shown on a log scale. The pump beam is at 1046 nm, from a semiconductor 6 ps pulse width laser. The broadening to the "continuum" is as a result of many non-linear processes. Right: Post filtering spectrum, after IR cold mirror (blue) and glass filters (red).

The high speed photodiode was the silicon PDM 400 from Becker and Hickl with a 400 ps full width at half maximum. The sample fluorescence photodetector was a single
photon counting module with an avalanche photodiode from Perkin Elmer, with a timing resolution of 350 ps. The electrical signals were amplified, digitised, counted and times registered on a TimeHarp 260 Pico TCSPC module from PicoQuant, the histogram was then processed on the computer.
2.5 Results and discussion

2.5.1 Manufacture

2.5.1.1 Manufacture using the lift-off method

A ZMW device is a metal film produced with periodic apertures allowing light to pass into the bottom of the apertures. In order to excite the ZMW a glass substrate is used. The lift-off process is often used, which produces a negative-tone pillar in an area clear of resist allowing metal to be deposited between each pillar. This can be achieved utilising PMMA’s positive-to-negative tone transition with increasing electron dose. The results produced are isolated metal films containing ZMW apertures. The isolation of small areas of metal film, improved the identification of ZMW under the optical microscope.

Initial work was conducted using a silicon substrate to find the optimum resist exposure parameters, and the maximum ZMW repeat period possible. Silicon is far less prone to capacitive charging than glass in an electron beam, which causes localised charge build-up on dielectric substrates. This can then generate a large static electric field, which causes beam deflection[24]. This disrupts the form of the pattern being written in an unpredictable manner, and changes the electron dose due to deflection to other regions.

The result of pillars produced in the centre of a positive tone area, cleared of resist is depicted in figure 2.13. The results on silicon are encouraging with clearly defined pillar features produced, with repeat periods up to 800 nm. The uniformity of the structure is good, showing that there is good adhesion to the surface and the structures are exposed correctly. With the 800 nm period structures the centre of the array is well formed, but the corners have a lower yield.
CHAPTER 2. ZERO MODE WAVEGUIDES

Figure 2.13: Negative tone pillar arrays for liftoff made on a silicon substrate with various period sizes, the resist was cleared in a positive tone manner around the negative features for all the periods with the exception of the largest period of 1000 nm where the resist dose was insufficient.

The 800 nm period is large relative to the wavelength of 450 nm, and the size of the pillar features which are 80 nm. Small diameters are important to reduce unwanted light transmission, through the ZMW[4]. For the pillars at a period of 1000 nm the resist was not fully cleared away between and around the pillars. The areas where resist appeared to have been cleared, had significant inter-pillar debris remained, which would affect the performance of the lift-off process.

ZMW test structures were made by depositing metal onto the high organised 400 nm pillars, illustrated in figure 2.13, the result is shown in figure 2.14. The ZMW side-walls
were poorly defined indicating that pillars with a larger period and with less order would produce results with more major defects. The larger period structures have a lower exposure dose due to a less effective proximity effect, and contained more unwanted residue around the pillar structures.

The centres of the ZMW pores also appear to have resist residue still present in some and a large variation in diameter. Electron beam instabilities from electrical interference or external vibration is likely to have affected the result. In addition there is also a clear vertically orientated astigmatism in the pores produced which enlarged the pillar area but this is not likely to have caused all of these defects and the electron dose was clearly high enough to ensure the onset of negative tone features in resist. The adhesion of the pillars appears very high even after soaking in acetone overnight there are still traces present in the middle of some pores.

Figure 2.14: SEM image of the result of depositing metal over a 400 nm array as shown in figure 2.13 and subsequent removal of the pillars in acetone. Larger features are a result of pillars which fell onto their sides. The pore sidewalls are not well defined and are very rough in appearance. The SEM image was processed to reduce noise and improve contrast.

The ZMW application utilises a glass substrate. The lift-off method was replicated on a glass substrate, figure 2.15 was the first test conducted. The resist was spun directly onto glass and written by electron beam. The pillars were not exposed in the same manner as on silicon, which used the dose of 200 µC. The features on glass tended to fall in their positions, indicating a reduced adhesion to the surface or an insufficient exposure at the base of the pillar. Insufficient exposure close to the substrate would not allow the positive
to negative tone transition in the resist to complete. The average atomic mass of glass is lower compared to silicon, resulting in fewer backscattered electrons from the substrate. This should lower the exposure of the base of the pillars. Increasing the electron dose by a factor of 64 to 12800 $\mu$C did improve the result in figure 2.15. There were still a significant number of fallen features, even the centre of the exposure, which has the highest dose due to the proximity effect. This indicates there is an pillar-substrate adhesion problem. The straight pillar profile is shown from pillars, which had fallen, and is illustrated in the enlargement. In the event of insufficient exposure feature-narrowing down the profile would be expected. This however is not observed.

![SEM images of pillars on glass and the poor adhesion to the substrate.](image)

**Figure 2.15:** SEM of pillars on glass and the poor adhesion to the substrate. Increasing the electron dose from 200 $\mu$C by a factor of 64 to 12800 $\mu$C did help but there still insufficient adhesion to the glass substrate. In addition to that improvement there was significant inter-pillar debris which would disrupt metal deposition.

When larger areas were trialled the local charge build-up on the glass surface caused large distortions to distribution of features. A layer of silver deposited over the resist
greatly reduced charging. Adding a layer of silver requires a selective chemical etchant to remove it after exposure, to allow development and further processing. Early in the project the author was not aware of a non-acidic silver etchant, such as those used later containing potassium ferricyanide and sodium thiosulfate in section 4.3.4. The etchant used at the time was dilute 0.1 M nitric acid. This is a very aggressive acid, which damaged the pillars produced, this is illustrated in figure 2.16.

![Figure 2.16: SEM of large array of 400 nm period structures on glass with and without a top layer of silver used to reduce local charging on the glass substrate, on the right and left respectively.](image)

**Left:** Significant distortion from charging is seen without a silver layer, **Right:** with a silver layer much less feature placement distortion is present but the silver etchant has damaged the features, seen as black points. The lack of development allowed the aggressiveness of the etch to be more clearly seen.

### 2.5.1.2 Manufacture utilising an etch mask

To avoid the use of toxic silane-based adhesion promoters[25], a new method was developed utilising aluminium etching through a resist etch mask. A glass slide was coated with 50 nm of aluminium and PMMA resist was spun on top. Exposing the PMMA to a low electron dose created pores in the resist. The conductive aluminium prevented localised charge accumulation and pattern distortion. An advantage to this technique was that the ZMW could be more widely separated, reducing the probability of coupling to allow enhanced transmission[4].

The main disadvantage to this method was the relatively large feature size created, of 100–200 nm. This is significantly larger than the 80 nm pillars produced from metal lift-off.
The sidewall profile of the ZMW produced was sloped as opposed to the conventional vertical profile, this is apparent through the gradient in brightness inside the ZMW pores in figure 2.17 A–B and D–E. The ZMW produced have a smooth profile, in contrast to the rough and damaged side-walls produced in the lift-off method on silicon shown previously in figure 2.14.

Figure 2.17: SEM of two different sizes of zero mode waveguides made via chemical etching. The pore sizes were 100 nm for the 10 nm design size A–C and 200 nm for the 40 nm design size D–F. C & D are on a tilt to examine the etch profile. Etching was conducted for 285 s.
2.5.2 ZMW optical properties

Chromophores inside ZMW are predicted to have an enhanced emission rate and be more spatially isolated than is normally possible. This advantage will allow single molecule spectroscopy to be conducted with solution concentrations closer to biological conditions, in the micromolar regime.

2.5.2.1 Fluorescence lifetime inside ZMW

Fluorescence lifetime reduction has been linked in the literature with fluorescence emission enhancements[26, 27] and signal to noise ratio improvements[5]. The choice of dye[28], the power of the excitation objective[28], the laser power[28] and the environment inside the ZMW[7] have an effect on the fluorescence emission efficiency.

The fluorescence lifetime of rhodamine 110 is greatly depressed inside the ZMW from 4.1 ns[29], to 530 ps. This is a reduction by a factor of 8 and is close the limit of the resolution of the TCSPC equipment. The timing resolution is limited by the laser pulse width (350 ps), photodiode rise time (200 ps) and pulse width (400 ps), avalanche photodiode timing resolution (350 ps) and the accuracy of the electronic timing measurement (25 ps). Curve fitting was successful using least squares regression, producing a good fit with \( \chi^2_{\text{red}} = 1.06 \).

In order to determine the effect of this lifetime change on the molecular brightness, and any changes in emission efficiency, the technique of FCS was utilised.
2.5.2.2 FCS measured confocal volume and number of molecules

As was detailed in section 1.7, FCS is a single molecule sensitive technique to establish the molecular brightness and the diffusion half-life inside the focal volume. Since the diffusion rate coefficients of many dyes are known, the diffusion half-life gives a measure of the average radius of the confocal volume. To calculate molecular brightness the emission intensity and correlation amplitude is recorded, the inverse of which is related to the number of molecules allowing the counts per molecule to be estimated. For measurements at constant concentration the relative number of molecules is an independent measure of the relative size of the confocal volume. This is important as the precise geometry inside the ZMW pore is difficult to measure from SEM images and autocorrelation curve fitting models assume three dimensional Gaussian structure which is only an approximation. Since the confocal volume of the standard control is well described by the fitting equations in the literature relative numbers of molecules may be estimated reliably, to deduce the relative confocal volume of a ZMW device.
2.5. RESULTS AND DISCUSSION

Figure 2.19: Correlation graph of rhodamine 110 inside ZMW at different power levels, compared to a confocal microscope control at 210 $\mu$W. The correlation amplitude indicates there are 1.51, 4.10 and 7.16 molecules in the 210 $\mu$W ZMW, 61 $\mu$W ZMW and 210 $\mu$W confocal control respectively.

Calibration measuring the confocal volume in solution found it to be $2.92 \times 10^{-15}$ L. In a ZMW device measured at high power (210 $\mu$W) the relative number of molecules was a factor of 4 lower than the control without a ZMW device. A naïve estimate of the ZMW confocal volume is $2.92 \times 10^{-15} \text{ L} / 4 = 7.29 \times 10^{-16}$ L, this is assuming the excitation volume profile is common between the control and the ZMW. The measured number of molecules were 1.99 and 7.95, from the ZMW at 210$\mu$W and the control, respectively. This was extracted from figure 2.19 by curve fitting. The confocal volume of the ZMW at 210 $\mu$W was calculated to be $3.76 \times 10^{-16}$ L. The fit data used is given in table 2.1 and equations 2.5.1 and 2.5.2 are replicated from section 1.7.1 below,

\[
\omega_0^2 = 4D\tau_{\text{diff}} \tag{2.5.1}
\]

\[
V_{\text{eff}} = \frac{\pi}{3}S\omega_0^3 \tag{2.5.2}
\]

There appears to be a contradiction in the measured confocal volume of the ZMW and the number of molecules present, the confocal volume is a factor of 8 smaller but with only a factor of 4 fewer molecules present. This seems to imply that there is factor of two, greater local concentration of molecules around the ZMW than, in the confocal control at the same concentration. This could be attributed to surface adsorbed molecules, restricted
diffusion or local confinement by the high laser intensity.

This conclusion appears strange until one considers the lower power 61 µW data, which when fit with a single 3D volume and triplet, has large structured residuals in figure 2.20 top, with relatively large error estimates in table 2.1. The triplet state lifetime is also uncharacteristically long at 45–50 µs compared to 2–3 µs in the confocal and high power ZMW.

Table 2.1: Table of fit parameters for a 100 nM solution of rhodamine 110 in a confocal volume and in ZMW, illuminated at 200 µW and 61 µW. Modelled with a single component diffusion model with a triplet correction.

<table>
<thead>
<tr>
<th></th>
<th>200 µW error</th>
<th>61 µW error</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZMW fit n_total</td>
<td>± 0.0009</td>
<td>± 10.6050</td>
</tr>
<tr>
<td>conf. fit tfrac</td>
<td>± 0.0001</td>
<td>± 0.5660</td>
</tr>
<tr>
<td>ZMW fit τ_trip</td>
<td>± 3.5465</td>
<td>± 46.5708</td>
</tr>
<tr>
<td>ZMW fit τ_diff</td>
<td>± 0.0497</td>
<td>± 1.1543</td>
</tr>
<tr>
<td>S offset</td>
<td>± 2.7033</td>
<td>± 3.0000</td>
</tr>
<tr>
<td></td>
<td>± 0.0016</td>
<td>± 0.0001</td>
</tr>
</tbody>
</table>

* a n_total, total number of chromophores.
* b tfrac, triplet fraction.
* c τ_trip, triplet state lifetime.
* d τ_diff, diffusion lifetime.
* e S, confocal ratio Z₀/ω₀.
* f offset, a correction for non zero correlation at infinity.

### 2.5.2.3 Parameter extraction from ZMW at excitation low power

A model with two diffusing components is a closer fit to the 61 µW ZMW data, than single component diffusion with triplet correction this is illustrated in figure 2.20 top. The fit residuals are shown blue in figure 2.20 (bottom) are near zero between 1–10⁻⁴ s. The two component fit has a triplet lifetime of 19 µs, which is quite uncharacteristic considering the 2–3 µs found from the confocal control and the fit to 200 µW ZMW. The fit residuals are also fairly structured at short time, between 10⁻⁷–10⁻⁶ with a clear positive trend, implying that the triplet aspect to the fit is not representing the data well.

This suggests there could be three diffusing components instead of only two and to remove the triplet contribution, the fit residuals corresponding to this are shown in figure 2.20 bottom in red. The three component model’s fit residuals are closer to the ideal with no clear trend with a zero mean throughout the range, within experimental error. The
individual component parameters for the three component model shown in table 2.2, have the same order of magnitude as those the confocal control and from the ZWM at 200 µW, further supporting the likelihood of a three component model.

In the three component model the long diffusion component implies there is a state where the dye is relatively immobile, possibly adhered onto the surface of the aluminium with a decay time of 33 ms. However, this state only accounts for 7% of the total number of molecules. The intermediate component at 0.31 ms is close to the confocal control of 0.15 ms. This implies there is light leaking into the far side of the device outside of the ZMW pore itself in a diverging manner hence the longer residence time. From the relative numbers in each component of 49.9% and 42.7% inside and outside the pore respectively the axial extent of the excitation is likely to be more limited than the radial extent, this accounts for the reduced number of molecules and larger residence time. It is known that ZMW attenuate the excitation intensity, so it is consistent that the axial extent is more limited. Though that same property could be attenuating the signal from excited fluorescence, from a far greater excitation volume on the far side of the ZMW volume.

Table 2.2: Table of fit parameters for a 100 nM solution of rhodamine 110 in a ZMW, illuminated at 61 µW. Modelled with a two and three component diffusion model with and without a triplet correction respectively.

<table>
<thead>
<tr>
<th>61 µW ZMW 2 diff. comp. &amp; trip.</th>
<th>61 µW ZMW 3 diff. comp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_{\text{total}}$ a</td>
<td>7.0803</td>
</tr>
<tr>
<td>$\tau_1$ (ms) b</td>
<td>0.2470</td>
</tr>
<tr>
<td>$\tau_2$ (ms) b</td>
<td>32.046</td>
</tr>
<tr>
<td>$\tau_{1,\text{frac}}$ c</td>
<td>0.8683</td>
</tr>
<tr>
<td>$S^d$</td>
<td>3.0000</td>
</tr>
<tr>
<td>$\tau_{\text{trip}}$ (µs) e</td>
<td>19.233</td>
</tr>
<tr>
<td>$t_{\text{frac}}$ f</td>
<td>0.3820</td>
</tr>
<tr>
<td>offset g</td>
<td>0.0008</td>
</tr>
<tr>
<td>$n_1$ a</td>
<td>6.1481</td>
</tr>
<tr>
<td>$n_2$ a</td>
<td>0.9322</td>
</tr>
<tr>
<td>$n_3$ a</td>
<td>0.3101</td>
</tr>
<tr>
<td>$\chi^2_{\text{red}}$</td>
<td>1.7058</td>
</tr>
</tbody>
</table>

a $n_{\text{total}}, n_n$ total or component $a$ chromophore quantity.
b $\tau_n$, diffusion lifetime of component n.
c $\tau_{n,\text{frac}}$, number fraction of component n.
d $S$, confocal ratio $z_0/\omega_0$.
e $\tau_{\text{trip}}$, triplet state lifetime.
f $t_{\text{frac}}$, triplet fraction.
g offset, a correction for non zero correlation at infinity.
CHAPTER 2. ZERO MODE WAVEGUIDES

Figure 2.20: Residuals from fitting the correlation curve from a ZMW illuminated at 61 $\mu$W, with models with 1–3 diffusing components, illustrated in green, blue and red respectively. The single component fit model included a triplet correction. The two component model residuals are less structured throughout and especially where the data has least noise between $10^{-4}$ and $10^{-1}$ s. The single component model has clear, large amplitude structure in the residuals.

Figure 2.21: Residuals from fitting the correlation curve from a confocal microscope and ZWM illuminated at 200 $\mu$W with a one component three dimensional model and triplet correction shown as red and blue respectively. The noise is the comparable to any apparent structure implying a good fit.

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2.5. RESULTS AND DISCUSSION

Figure 2.22: Normalised correlation graph of rhodamine 110 inside ZMW at different power levels, compared to a confocal microscope control at 210 $\mu$W. Normalisation was done over an average of the first 6 $\mu$s where the curve has plateaued. The residence time is shorter by a power of ten for the ZMW device with 210 $\mu$W irradiation as compared to the confocal control. When a ZMW is used at lower power there appears to be some long lived close interaction with the metal surface which is lost due to photo-bleaching at high power.

An independent measure of the confocal volume can be found by interpreting the residence time, the $1/e$ time for the confocal control, and ZMW device illuminated at high power are; 200 $\mu$s and 50 $\mu$s respectively. This can be seen in the normalised FCS curves shown in figure 2.22. There is a factor of 4 between these two average decay times, which is consistent with the value found from curve fitting. This gives the same factor of 8 smaller confocal volume with twice the local concentration.

The long lived 33–1000 ms state, present at low power, appears to be a strong interaction. This time-scale is consistent with strong inter-molecular forces, but is only meta-stable, which rules out chemical bonds. This interaction time is significantly longer than triplet dynamics allow, which occurs on the microsecond timescale for the dye, rhodamine 110. The interaction is likely via hydrogen bonding, since there are four hydrogen bonding sites: The iminium cation $\equiv$NH$_2^+$, the cyclic oxygen, the amine group $-$NH$_2$ and the carboxylic acid group $-$CO$_2$H. Aluminium on the ZMW surface forms a surface oxide layer 2–3 nm thick. This has negatively charged hydrogen bonding donors AlO$^-$, and terminal hydroxide AlOH hydrogen bond acceptors. The bond strength estimated for this interaction is between 32–120 KJmol$^{-1}$[30, 31], which is between 12.8 and 48 times thermal energy RT at room temperature[32], at 2.5 KJ mol$^{-1}$. High laser power causes additional heating, providing the energy to break the formed hydrogen bonds so the
surface-bound hydrogen bonded state of rhodamine 110, is no longer observed. However, a rapid equilibrium could be the cause of the localised increase in concentration, compared to the confocal control.

![Diagram showing chemical structure and hydrogen bonding sites of rhodamine 110](image)

Figure 2.23: Chemical structure showing the four hydrogen bonding sites of rhodamine 110, depending on the specific atom pair in the hydrogen bond, the strength is between 8–40 KJ mol\(^{-1}\) per bond. So the largest surface interaction force is between 32–120 KJ mol\(^{-1}\) which is 10–33% of a typical chemical bond strength. The corresponding hydrogen bonding sites on the aluminium’s surface oxide layer are also shown below.

There appears to be great variability in the literature, with many studies not observing the long lived states[1, 3], while it is very obvious in others[1, 11, 33].

This effect was also seen in a single gold NW[34] and on gold bow tie nanoantenna[35]. In these systems the higher the laser power, the lower the population of the long lived state[34, 35]. This was explained by an adhesion mechanism, followed by photo-bleaching[34]. In another study, they added surfactant to reduce the surface interaction, which removed the long lived state from the results[36]. Though adding surfactant changes the density, viscosity and therefore transit times and measurement of the results. In addition surfactant is not generally compatible with biological systems, where it can greatly disrupt protein folding[37]. Adding a surface coating to ZMW devices greatly reduces the brightness of fluorescence emitted from the ZMW[38], by reducing close surface-chromophore interaction[39, 40].

The long lived surface state has been observed in the literature, at low excitation powers[34, 35], but they have not suggested a mechanism by which the adhesion is occurring[34, 35].
2.5.3 FCS and TCSPC derived insight

The fluorescence enhancement $\eta_F$, for the ZMW devices is displayed in table 2.3. All $\eta_F$ values were less than unity when compared to the confocal solution FCS control with the minimum required fluorescent filtering. ZMW require an additional level of fluorescence filtering to remove device reflection, which is not normally required to perform a solution FCS measurement. A more informative test of the practicality of ZMW, compares the most suitable filter configurations for both the ZMW and control. This contribution is included in the light collection efficiency $\kappa$, term.

Table 2.3: Table comparing FCS derived properties of 100 nM rhodamine 110 in a confocal control and a ZMW device at 210 $\mu$W and 61 $\mu$W.

<table>
<thead>
<tr>
<th></th>
<th>N mol.</th>
<th>Brightness</th>
<th>CPM</th>
<th>Rel. N mol.</th>
<th>$\eta_F$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a.u.)</td>
<td>(KHz)</td>
<td>(KHz mol$^{-1}$)</td>
<td>(a.u.)</td>
<td>(a.u.)</td>
</tr>
<tr>
<td>Control 210uW</td>
<td>7.16</td>
<td>571.59</td>
<td>79.80</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>ZMW 210uW</td>
<td>1.51</td>
<td>76.58</td>
<td>50.75</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>ZMW 61uW</td>
<td>4.10</td>
<td>46.28</td>
<td>11.29</td>
<td>0.57</td>
<td>0.14</td>
</tr>
</tbody>
</table>

$^a$ N mol, is the number of molecules.
$^b$ CPM is the count rate per molecule.
$^c$ Rel. N mol., is the relative number of molecules.
$^d$ $\eta_F = \frac{CPM^*}{CPM}$, the relative CPM.

To determine if there is any fluorescence enhancement $\eta_F$ experimental values are inserted into the following relations[41],

$$\eta_F = \frac{CPM^*}{CPM}$$  \hspace{1cm} (2.5.3)

$$\eta_F = \frac{\kappa^* k_{rad}^* \tau_{rad}^* I_{ex}^*}{\kappa \kappa_{rad} \tau_{rad} I_{ex}} \text{ when, } (I_{ex} \ll I_{saturation})$$  \hspace{1cm} (2.5.4)

$$\eta_F = \frac{\kappa^*}{\kappa_{rad}} \text{ when, } (I_{ex} \gg I_{saturation})$$  \hspace{1cm} (2.5.5)

Where $^*$ denotes in a device, CPM is the counts per molecule emitted when fluorescence is excited, $\kappa$ is the light collection efficiency (related to directionality of emission), $k_{rad}$ is the radiative emission rate and $I_{ex}$ is the excitation intensity. The ZMW pore attenuates the $I_{ex}$ as a way of localising the excitation volume beyond the diffraction limit. Though the
fluorescence lifetime was decreased by a factor of eight it is not clear from TCSPC if the chromophore brightness was affected, as that is related to $k_{\text{rad}}$ and $\kappa$ but those factors can be examined using FCS.

The discussion of excitation power in section 2.5.2.3 found that ZMW excited at a power of 61 $\mu$W behaved quantitatively different to ZMW excited at 210 $\mu$W. Therefore it is likely that the ZMW at 61 $\mu$W is below the saturation threshold $I_{\text{saturation}}$. By extension, 210 $\mu$W must be above the $I_{\text{saturation}}$ threshold and can be set equal to equation 2.5.3. This yields the following expressions,

$$\eta_f = 0.64 = \frac{\kappa^* k_{\text{rad}}^*}{\kappa k_{\text{rad}}},$$

inserting into the equation for $\eta_f$ above saturation gives,

$$\eta_f = 0.14 = 0.64 \times \frac{\tau_{\text{decay}}^* I_{\text{ex}}^*}{\tau_{\text{decay}} I_{\text{ex}}},$$

rearrangement and insertion of $\tau_{\text{decay}} = 4.1$ ns and $\tau_{\text{decay}}^* = 530$ ps, gives,

$$1.7 = \frac{I_{\text{ex}}^*}{I_{\text{ex}}}.$$  

The main factor reducing the ZMW efficiency is the fluorescence lifetime $\tau_{\text{decay}}$ decrease, by a factor of 8. A modest reduction in product of radiative rate and light collection efficiency at 0.64 also reduces the ZMW efficiency. These effects are only partially offset by the modest increase in excitation intensity from the local SP at the interface, with a factor of 1.7. The net result is a fluorescence inhibition inside ZMW as opposed to an enhancement.
2.6 Conclusions and outlook

ZMW were successfully fabricated, characterised and tested. Fabrication was achieved by coating an aluminium layer onto glass, spin-coating a resist layer on top, and patterning the resist using electron beam lithography. The resist pattern defined an etch mask, and then using wet chemical etching a hole was defined in the aluminium film on a glass substrate. Limited progress was obtained using the lift-off method, because of the issues surrounding capacitive charging of the glass substrate, in the electron beam. Characterisation showed that features of between 100–200 nm could be created reliably on periods of several microns, which were ideal for the ZMW application as they were smooth and regular.

TCSPC and FCS gave an insight into the fluorescence quenching inside ZMW, which contradicts the popular literature perspective\[5, 6\]. The reduction in fluorescence enhancement was primarily caused by the reduction in the fluorescence lifetime also contrary so some literature reports\[26, 42, 43\]. The choice to compare the normal low level of fluorescence filtering used in solution FCS, to the required high level of filtering used in ZMW was to give a more realistic comparison. Significant filtering is required with ZMW because of the very strong reflection of the excitation laser from the ZMW devices. In some cases it was not possible to remove the laser light entirely, while still obtaining acceptable signal to noise ratios. By contrast, control solution measurements do not require such heavy filtering, and can therefore produce far greater signal to noise ratios.

The primary goal of confining light to a nanoscale volume to achieve higher concentration SM FCS was successfully achieved. The volume was only a factor of 7.8 smaller than the control, and there was a factor of two higher local concentration than would normally be expected around the ZMW. Studying the ZMW at low power showed there were two likely additional components, molecules hydrogen bonding to the surface and transmitted light exciting fluorescence in the solution above. These components are thought to contribute at higher power excitation in the form of a rapid equilibrium, which increases the local concentration. The limited success of the ZMW approach and the loss of the electron beam lithography equipment has motivated further chapters to explore a different structure. A self assembled hexagonal structure, of vertically aligned silver NWs, also on a glass substrate. In order to make this structure the first stage was to create an alumina template,
as detailed next in section 3.1.
BIBLIOGRAPHY


CHAPTER THREE

POROUS ALUMINA TEMPLATES
3.1 Introduction

Anodization is the process of oxidising a material with an applied voltage using an oxidising agent, which is often acidic. Anodizing aluminium creates aluminium oxide, there are two different types of anodisation which both create unique structures, porous and barrier type. Barrier type is a planar oxidation process in mild acidic conditions and voltage, which has an exponentially decaying rate as a function of time, this has applications in capacitors because of the oxide layer is highly insulating[1].

Porous type anodisation creates cylindrical pores of aluminium oxide, which are formed using strongly oxidising, organic acids and high voltages. The alumina produced using porous type anodisation is otherwise known as porous anodic alumina, and is abbreviated to \textbf{PAA}. A schematic of the hexagonal close-packed structure of PAA is illustrated in figure 3.1.

![Figure 3.1: Schematic of the target hexagonal PAA structure mounted directly on a glass substrate.](image)

The diameter of the pores produced inside PAA and the inter-pore distance are a function of the acid electrolyte utilised and the applied potential. The most widely used acids are sulfuric, oxalic and phosphoric acid, which produce structures with a diameter of 10–70 nm[2–5], 30–80 nm[6, 7] and 100–500 nm[3, 8], respectively. The inter-pore distances for these structures are 30–100 nm[2–5], 60–140nm [6, 7] and 150–700 nm[3, 8], respectively. The large surface area of PAA is also used in capacitors[1], and to create strong bonding surfaces for paint and adhesives[9]. When PAA is heated in boiling water with coloured dyes in the pores, they become sealed[10] inside, producing durable colouring of aluminium. The application of interest is to use the porous structure of PAA, as a template to create metal NWs. Free-standing vertically aligned NWs can be produced,
which are anchored onto the substrate.

The anodising reaction involves the following initial step, which converts aluminium to the ionically porous aluminium oxide, in acidic media with an applied voltage[11],

\[ 2 \text{Al} + 3 \text{H}_2\text{O} \rightarrow \text{Al}_2\text{O}_3 + 6 \text{H}^+ + 6 \text{e}^- \]  

(3.1.1)

An oxidant added to the system consumes the free electron and is itself reduced; example oxidants include oxalic and sulfuric acids. In order to dissolve the bulk aluminium, first aluminium ions are formed as in equation 3.1.2. These aluminium ions may be converted to aluminium oxide as in equations 3.1.3 and 3.1.4[11],

\[ \text{Al} \rightarrow \text{Al}^{3+} + 3 \text{e}^- \]  

(3.1.2)

\[ 2 \text{Al}^{3+} + 3 \text{H}_2\text{O} \rightarrow \text{Al}_2\text{O}_3 + 6 \text{H}^+ \]  

(3.1.3)

\[ 2 \text{Al} + 6 \text{OH}^- \rightarrow \text{Al}_2\text{O}_3 + 3 \text{H}_2\text{O} + 6 \text{e}^- \]  

(3.1.4)

The aluminium ions produced may also be converted to acid-soluble aluminium hydroxides as in equations 3.1.5, 3.1.6 and 3.1.7[11],

\[ \text{Al}^{3+} + \text{H}_2\text{O} \rightarrow \text{AlOH}^{2+} + \text{H}^+ \]  

(3.1.5)

\[ \text{Al}^{3+} + 2 \text{H}_2\text{O} \rightarrow \text{Al(OH)}_2^+ + 2 \text{H}^+ \]  

(3.1.6)

\[ \text{Al}^{3+} + 3 \text{H}_2\text{O} \rightarrow \text{Al(OH)}_3 + 3 \text{H}^+ \]  

(3.1.7)

By chance these reactions may occur faster at some locations than others. Over time, these reaction centres appear to repel one another, forming an overall hexagonal close packed structure. The mechanism by which this occurs is disputed, mechanical stress[12] created during the process and electrical charge at the reaction centres are two leading theories. It has been suggested that both mechanisms play a part in the structure’s organisation[13]. During anodisation the pore organisation over time has prompted the
development of multi-stage anodization processes[14], where at each stage the PAA is more organised than the last. The PAA is then selectively removed, leaving dimples as an imprint of the previous structure on the exposed aluminium surface. The increasing structure of the PAA film after multiple stages is depicted schematically in figure 3.2.

![Figure 3.2: Schematic of the standard two stage anodising process.](image)

The disadvantage of two stage anodization is the technical challenge of producing ultra high purity aluminium, with very low roughness with a large thickness[15]. Physical vapour deposition methods of coating as described in section 2.3.4 are only well suited to thin films. Producing thick films pose greater challenges, because the vacuum quality has to be maintained for longer durations while coating. Sample heating also increases over longer coating periods, leading to sample crystallisation, and oxidation when air is readmitted into the chamber. All of these effects can reduce the purity and increase the surface roughness of the aluminium coatings and lead to oxide formation and other contamination.

Some of these limitations can be circumvented using expensive pre-patterning techniques such as ion beam milling and electron beam lithography, to define the desired structure on the aluminium surface prior to anodisation. These methods produce more ordered PAA in a single step than is normally possible, avoiding the need for the anodisation second step[16, 17]. Although effective, these are not suitable for large areas or large scale production. The costs of these techniques are prohibitively high due to their low throughput, high capital cost and complex operation. Patterning the aluminium can be accomplished using an ultrahard press made of silicon carbide, which is structured with the negative image of the desired pattern. These have proven effective on bulk metal, but the force required and fragility of thin films limits their applicability outside of bulk
aluminium[18].

3.2 Experimental

3.2.1 Atomic force microscopy

Atomic force microscopy (AFM) is a type of scanning-probing microscopy, where a sharp tip is scanned across a sample surface. The sample surface is detected by monitoring the laser reflection from the top, flat and reflective surface of the tip. Laser reflection is focused onto a four photodiode array, known as a quadrature photodiode, the ratios of the intensities from these photodiode report of the angle of the tip. Scanning is accomplished using a pair of precise piezoelectric actuators. A simplified schematic of the experimental apparatus is shown in figure 3.3 below.

![Figure 3.3: Schematic of the AFM experimental apparatus.](image)

3.2.2 Metal deposition

3.2.2.1 Sputtering

The first method trialled was of sputtering, which was previously described in section 2.3.4. The main parameters important to radio frequency sputtering are the sputtering gas composition, pressure, sample position, power input into the plasma, sputtering duration, sample cooling time during and after sputtering. Sputtering gas composition is generally a noble gas such as argon, except when depositing metal oxides from a metal target, where oxygen is added to the plasma. Sputtering gas pressure partially determines the density of sputtered atoms to coat the sample, unionised gas contributes to collisional cooling.
CHAPTER 3. POROUS ALUMINA TEMPLATES

of the sample. Sample position determines the density of plasma at the sample, this is caused by the approximately inverse square fall-off of the plasma density. Sputtering power determines the proportion of the sputtering gas, which is ionised and capable of atomising a sputtering target to coat the sample. Sputtering duration determines the thickness of the coating produced and indirectly influences the sample temperature. Sputtering in short time intervals, with time allowed for cooling, can lower the sample temperature.

Many of these parameters were independently varied for aluminium sputtering are illustrated in table 3.1 below.

3.2.2.2 Evaporation

An alternative method of aluminium deposition is vacuum evaporation. During aluminium evaporation the evaporation chamber is at high vacuum, around $1 \times 10^{-7}$ mbar reducing the concentration of contaminant gases such as oxygen and water. This increases the probability of aluminium atoms, directly condensing onto the sample without contamination.

Large tungsten evaporation boats or very tight custom made helical coils were produced. The large surface area of the helical coils allowed large quantities of molten aluminium to wet and evaporate. High currents, were able to liquefy and evaporate up to 0.4 g of 99.999 %wt aluminium pellets, and create films 1000–1500 nm thick depending on sample position. These films were deposited rapidly in under four minutes, at 4.2–6.3 nm s$^{-1}$, up to sixty times faster than the 0.1–1.5 nm s$^{-1}$ from sputtering.
Table 3.1: Table of sputtering parameters explored, position is the sample position in chamber, cooling time is under high vacuum (HV) or argon (Ar) or nitrogen (N\textsubscript{2}), cooling steps are the number of pauses during and after sputtering.

<table>
<thead>
<tr>
<th>Ar P (mbar)</th>
<th>Base P (mbar)</th>
<th>Power (W)</th>
<th>Sput. time (min)</th>
<th>Sput. rate (nm/s)</th>
<th>Thickness (um)</th>
<th>Position in chamber</th>
<th>Cooling time (min)</th>
<th>Cooling steps</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1E-03</td>
<td>3E-05</td>
<td>45</td>
<td>30</td>
<td>Bottom</td>
<td>5 HV</td>
<td>3</td>
<td>Oxidised, 5–10 nm RMS roughness.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>1.25E-06</td>
<td>70</td>
<td>62</td>
<td>3.00</td>
<td>11.16</td>
<td>Bottom</td>
<td>1</td>
<td>Bad adhesion, sonication damage.</td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>1.75E-06</td>
<td>30</td>
<td>10</td>
<td>Bottom</td>
<td>10 HV</td>
<td>1</td>
<td>Good, non-uniform thickness.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7E-04</td>
<td>4E-06</td>
<td>20</td>
<td>70</td>
<td>0.50</td>
<td>2.10</td>
<td>Bottom</td>
<td>1</td>
<td>Oxidised.</td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>2E-06</td>
<td>50</td>
<td>95</td>
<td>Bottom</td>
<td>10 HV</td>
<td>1</td>
<td>Oxidised.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>5E-06</td>
<td>50</td>
<td>120</td>
<td>0.30</td>
<td>2.16</td>
<td>Bottom</td>
<td>40 HV</td>
<td>1</td>
<td>Oxidised, acid cleaning and longer cooling no help.</td>
</tr>
<tr>
<td>1E-03</td>
<td>2E-05</td>
<td>40</td>
<td>120</td>
<td>Bottom</td>
<td>20 HV</td>
<td>1</td>
<td>Oxidised, last to use resist adhesive.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>7E-06</td>
<td>40</td>
<td>50</td>
<td>Bottom</td>
<td>20 HV</td>
<td>1</td>
<td>Oxidised, renovated system.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>7E-06</td>
<td>40</td>
<td>50</td>
<td>Middle</td>
<td>20 HV</td>
<td>1</td>
<td>Good, slow rate.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>7E-06</td>
<td>40</td>
<td>300</td>
<td>Middle</td>
<td>20 HV</td>
<td>1</td>
<td>Oxidised.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>6E-06</td>
<td>40</td>
<td>25</td>
<td>Middle</td>
<td>50 HV</td>
<td>1</td>
<td>V. oxidised, cooling too long in vacuum.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>1E-05</td>
<td>20</td>
<td>251</td>
<td>Bottom</td>
<td>20 HV</td>
<td>1</td>
<td>Oxidised, non-uniformly oxidised.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>8E-06</td>
<td>30</td>
<td>185</td>
<td>0.14</td>
<td>1.54</td>
<td>Bottom</td>
<td>10 HV</td>
<td>3</td>
<td>Oxidised, improved, non-uniformly oxidised.</td>
</tr>
<tr>
<td>1E-03</td>
<td>2E-05</td>
<td>80</td>
<td>15</td>
<td>Top</td>
<td>5 HV</td>
<td>1</td>
<td>Good, short duration.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>1E-05</td>
<td>80</td>
<td>60</td>
<td>Top</td>
<td>5 HV</td>
<td>4</td>
<td>Good, multistage, top chamber HV cooling.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>9E-06</td>
<td>100</td>
<td>15</td>
<td>Top</td>
<td>10 HV</td>
<td>1</td>
<td>Good, chamber baked out.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>9E-07</td>
<td>100</td>
<td>90</td>
<td>Top</td>
<td>10 Ar</td>
<td>1</td>
<td>Good, rapid Ar cooling.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>5E-07</td>
<td>100</td>
<td>210</td>
<td>Top</td>
<td>10 Ar</td>
<td>6</td>
<td>Good.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2E-03</td>
<td>1E-06</td>
<td>100</td>
<td>150</td>
<td>Top</td>
<td>10 Ar</td>
<td>3</td>
<td>Oxidised, sputtered too long.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>5E-07</td>
<td>100</td>
<td>30</td>
<td>Top</td>
<td>10 Ar</td>
<td>1</td>
<td>Good.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3E-03</td>
<td>9E-07</td>
<td>100</td>
<td>180</td>
<td>Top</td>
<td>10 Ar</td>
<td>1</td>
<td>Oxidised.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>1.8E-06</td>
<td>100</td>
<td>30</td>
<td>Top</td>
<td>10 Ar</td>
<td>1</td>
<td>Oxidised.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>3E-06</td>
<td>100</td>
<td>30</td>
<td>Top</td>
<td>30s HV, 20 Ar</td>
<td>1</td>
<td>Oxidised, HV exposure contaminated surface.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>2E-06</td>
<td>100</td>
<td>215</td>
<td>Top</td>
<td>5 Ar</td>
<td>43</td>
<td>V. oxidised, white, low reflectivity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E-03</td>
<td>5E-06</td>
<td>100</td>
<td>120</td>
<td>Top</td>
<td>5 HV</td>
<td>1</td>
<td>V. oxidised, white, low reflectivity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8E-4</td>
<td>1E-05</td>
<td>80</td>
<td>20</td>
<td>Top</td>
<td>3 HV</td>
<td>1</td>
<td>Good, system cleaned, low Ar sputtering pressure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8E-4</td>
<td>4E-05</td>
<td>80</td>
<td>10</td>
<td>Top</td>
<td>3 HV</td>
<td>1</td>
<td>Good, chamber baked out.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8E-4</td>
<td>6E-06</td>
<td>80</td>
<td>25</td>
<td>Top</td>
<td>3 Ar</td>
<td>1</td>
<td>Good, low Ar P sput. less cooling sensitive.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8E-4</td>
<td>6E-06</td>
<td>80</td>
<td>60</td>
<td>Top</td>
<td>3 Ar</td>
<td>1</td>
<td>Good, chamber baked, long spuit. time.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8E-4</td>
<td>4E-06</td>
<td>80</td>
<td>120</td>
<td>Top</td>
<td>3 Ar</td>
<td>1</td>
<td>Good, chamber baked, long spuit. time.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8E-4</td>
<td>6E-06</td>
<td>100</td>
<td>60</td>
<td>Top</td>
<td>none</td>
<td>1</td>
<td>Good, lower pressure Ar, cooling was not required.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2.3 Anodisation

Figure 3.4: Exploded schematic of the anodising setup, used to create PAA templates from a starting aluminium film on an insulating substrate. The circuit is achieved using a top contact and shorts to the solution are prevented by a seal in the centre of the sample. Cooling is ensured using the peltier device at the base of the sample.

Anodization of thin films on an insulating substrates requires electrical contact to the top aluminium surface. To achieve this a specially designed controllable flow cell was used, as is illustrated in figure 3.4. This is constructed from chemically inert polytetrafluoroethylene plastic with a hole inserted. Into this hole the sample was inserted and sealed against leaks using an o-ring, electrical contact and sample clamping was done at the sample corners, away from the flowing electrolyte. The electrical contact then connected to the high voltage supply via the sample holder. The other electrode made from the inert metal platinum, was positioned inside the cell in close proximity to the sample. Sample cooling was important in order to control the reaction rate and achieve more organised structures so below the sample holder a heatsink with an attached peltier cooler and water chiller cooling was used allowing temperatures as low as -20 °C. The sample temperature was monitored via a digital thermocouple fixed in the sample holder. To ensure the electrolyte uniformity with time in terms of local acid strength and temperature a peristaltic pump was used to circulate the electrolyte. Once the process was complete the electrolyte removal valve on the tubing at the base was opened to discharge the electrolyte and the sample and cell was rinsed with deionised water.

Labview custom software was used to control the duration and voltage applied during the anodisation from the attached constant voltage variable current supply. It allowed the
current density to be monitored as function of time as well as voltage, so any faults such as short circuit or the reaction’s completion could be detected.

To perform a multiple stage anodisation, the initial PAA layer must be removed selectively towards the underlying aluminium metal, potassium chromate $\text{K}_2\text{Cr}_2\text{O}_7$ mixed with 5% wt phosphoric acid is one such agent. The mixture is heated to 70 °C and the PAA sample is exposed to it for 30 minutes to ensure the reaction reached completion.

Once the PAA is fully formed on the substrate and anodisation is complete, which is when all of the available aluminium has been converted to PAA, the base of the pores must be etched to allow access to the underlying substrate. Etching with 5 wt% phosphoric acid for between 10–30 minutes operated at room temperature will allow access to the substrate inside the pores in the PAA template. The precise etching time varies according to the barrier layer thickness which is related to the applied potential in the anodisation process by the factor of 1.15 nmV$^{-1}$[19]. This process is called widening when the objective is to increase the pore diameter and pore opening when the objective is to allow access to the substrate, they are fundamentally the same process.
3.3 Results and discussion

3.3.1 Difficulties with sputtered films

Anodization requires very high purity aluminium in order to create the most ordered structures[20, 21], impurities such as aluminium oxide, formed during deposition, and contamination from copper, or other transition metals greatly reduce the organisation of the templates produced[20, 21]. Defects were produced as the PAA formed since the differences in solubility between the contaminants and the host aluminium.[20–22]. Ion transport through the porous oxide layer is also affected[20, 21], which in turn changes the local anodisation rate.

Differences in density and internal structure between the contaminant and the bulk aluminium result in a large roughness on the starting aluminium film. The degree of roughness is illustrated in figures 3.5 (Left) and 3.6 (Left) under the SEM and AFM respectively.

Figure 3.5: SEM of a sputtered aluminium film, after one step anodisation and after four step anodisation. Sputtered aluminium film has large surface features (100-200 nm), as a result of a long sputtering process. The single stage structure has been disrupted by this surface roughness, height variation is present. After four anodisation stages the ordering has deteriorated further and a two-tiered structure is visible. There is a large variation in pore size at all stages. The scale bars are all 200 nm.

After a single anodisation stage shown in figure 3.5 (Middle) the PAA has large differences in height and a disordered structure. After removing the PAA to exposure the aluminium below and re-anodising for a total of four stages the result has deteriorated further, in contrast to the reported trend of improvement[14]. In a multi-stage anodisation the first stage of PAA is removed by a selective chromate etch. This implies that the contaminants inside the aluminium film were etched during this etching step roughening
the patterned aluminium surface. The chromate PAA etch is only designed to be selective against etching aluminium metal.

![AFM of a aluminium film sputtered at 45 W, after annealing one hour 450 °C and after anodisation in oxalic acid at 40 V.](image)

Figure 3.6: AFM of a aluminium film sputtered at 45 W, after annealing one hour 450 °C and after anodisation in oxalic acid at 40 V. The original film defects are amplified by anodisation giving 16 nm RMS compared to 4 nm RMS in the starting material. This fault-amplification implies that the rough features are less reactive in the electrolyte than the base metal. Therefore they could contain an impurity such as an existing oxide layer or copper. Annealing did not improve the roughness significantly with both having 4 nm RMS roughness.

Steps were then taken to improve the quality of the coated aluminium. The sputtering target used was made of 99.999%wt aluminium, to be sure contamination had not occurred over time, the target was cleaned by strong acid and alkaline etching. This was then followed by sonication and multiple rinsing steps in deionised water. No improvement in the quality of the coating was observed. The sputter coating system was then stripped and cleaned, which lead to a minor improvement the level of vacuum and pumping speed. This did not ultimately improve the anodisation of the films produced.

The remaining factor was the combination of the quality of the vacuum and the low deposition rate. At a fixed level of vacuum, the concentration of contaminants is approximately constant, so a high deposition rate results in a less contaminated deposit. So a low deposition rate results in more contaminated aluminium film. Contamination sources include from surface adsorbed gases such as oxygen and water vapour and carbon from oil pumps and diffusion oil. The slow aluminium coating rate was unavoidable given the thickness uniformity required for anodisation. To ensure that the anodisation time for the sample was consistent, otherwise regions which anodised more quickly are corroded by the acidic conditions while anodisation is completed elsewhere. Coating uniformity could only be obtained with a large sample-sputtering target distance, the coating rate was slow even at the maximum sputtering power. Sample cooling intervals were able to control grain growth out of the plane of the film, which reduced roughness, this effect is demonstrated...
in figure 3.7. An annealing control was conducted to see the impact of grain growth on the number of defects. No significant differences were observed on the nanoscale by after annealing, or after anodisation of the annealed sample shown in figure 3.6 (Middle) and (Right) respectively. On the micron scale, grain enlargement produced grains protruding from the sample surface as illustrated by figure 3.7. This reduces the ability to produce a regular repeating pattern over a large area, so sample heating was reduced by inserting cooling periods.

Figure 3.7: Optical micrographs taken at 1000x magnification of the same sample, before and after annealing at 400° for 30 minutes under a nitrogen atmosphere. The grain growth has lead to a surface roughness increase, by growing out of the sample.

The aluminium film surface roughness was improved by these parametrisations, the lower surface roughness produced more ordered structures, but the elemental contamination from the low sputtering rate, still greatly disrupted the PAA structure limiting potential gains. The method of evaporation was initially discounted, since there were crucibles available, to coat a sample with the large quantities of aluminium metal required. In thin film evaporation, typically milligrams of material are evaporated at a time. What was required to produce micron thick films, was over a factor of 10 more aluminium to be evaporated at a rapid rate, without significant sample heating to a uniform thickness. This challenge is discussed in the next section.

3.3.2 Evaporated aluminium coating

Evaporation is a less complex experiment compared to sputtering, and coating aluminium requires no additional gases, there are also no inlet valves into the vacuum. In sputtering there are also additional points of failure, by contamination of the sputtering gas, and the sputtering plasma, sputtering-off impurities, from the instrument interior surfaces.
The level of vacuum was also higher, since in order to initiate sputtering the high vacuum valve was partially closed, to allow sputtering gas to be inlet, which is not required during evaporation. Typical evaporation starting vacuum was $1 \times 10^{-7}$ mbar, compared to the $1 \times 10^{-5}$ mbar typical of sputtering, once the high vacuum valve had been partially closed. The purity of the films produced was reflected in the large improvement of the anodisation results, which are sensitive to impurities at the 0.01% wt level[20–22].

Sputtering was abandoned as a method to make thick aluminium films because the deposition rate was relatively low when the sample was far from the sputtering target, a condition of obtaining the required thickness uniformity so that ordered structures could be obtained. The low deposition rate and reduced vacuum due to the requirement to partially close the valve to the high vacuum pump while sputtering is suspected to have lead to oxidation throughout the aluminium bulk as well as on the surface. The surface roughness was too great on the best sputtered samples to give ordered structures and attempts to conduct multiple stages did not overcome this because the scale of the roughness was too large.

Once large crucibles were fitted to the evaporator large currents were able to evaporate rapidly relatively thick coatings of 600–900 nm. This thickness is not enough for the electropolishing step often cited in the literature which rapidly consumes more than a micron of material and decreases the roughness in a field amplified dissolution of rough features to give a smoother surface. So this limited the maximum obtainable structural order produced via anodisation. However, since the wavelengths of light are factors of 4.5–15 larger than the structures minor disorder should be tolerable but will obviously degrade the quality of results possible.

The smallest template structures were obtained by anodising with sulfuric acid, as is outlined in the next section.

### 3.3.3 Sulfuric acid alumina templates

The small structures produced from sulfuric acid are well reported[3, 4], recently work has shown that relatively ordered structures may be obtained with pore sizes down to 17 nm[5]. Achieved by adding ethylene-glycol to the electrolyte mixture and cooling to
Adding ethylene glycol to phosphoric acid while anodising has also been shown to increase the rate of ordered structure formation and decreased the rate of attack of the aluminium substrate[8]. Utilising the limited aluminium thickness more efficiency than was previously possible to create ordered PAA is an ideal experiment. The 17 nm structural size is on the same order as the surface roughness, which is 2–5 nm, this may greatly deteriorate the structural order. The current densities of the first and second stage anodisation of aluminium on a glass substrate are shown in figure 3.8. They have the characteristic current spike and rapid decay at short time, where the barrier layer is formed and then the current density increases as the porous structure forms and the structure is reorganising. The eventual current slowdown occurs because the amount of the thin film aluminium remaining is reducing over time as it is consumed to become a PAA template.

![Current density versus time using H$_2$SO$_4$ (1.85 M, 16 wt %) and ethylene glycol (9.15 M, 50 wt %) at 19 V and 0°C, done in two stages. First stage of 9 minutes 30 seconds, and second of 5 minutes. The AFM of the alumina template is shown in figure 3.8.](image)

An AFM of the structures produced is shown in figure 3.9 which has pores a mean of 15 nm in diameter in a relatively organised hexagonal close-packed arrangement though it is clear that there are many defects, primarily from the starting aluminium surface structure. The disruption is evident in the range of pore sizes produced which is 8–20 nm a variation of 30–50 % about the mean. There is a frequent omission of a pore from the hexagonal unit cell, creating distorted pentagonal regions these appear to be formed where there are a higher density of surface defects in the starting surface. The anodisation time could have been insufficient, due to the limited aluminium thinkness since the order improves with
longer anodisation duration.

Widening of the pores was done for a relatively short time to allow easier visualisation under AFM and in the application that is required to obtain wires attached onto the substrate surface below.

![AFM image of an alumina template made using H₂SO₄ (1.85 M, 16 wt %) and ethylene glycol (9.15 M, 50 wt %) at 19 V and 0°C, done in two stages. First stage of 9 minutes 30 seconds, and second of 5 minutes. The pores were then widened in H₃PO₄ (0.1 M) for 5 minutes. The anodisation current density curve is shown in figure 3.8. The pores produced were in the range 8–20 nm (15 nm).]

The opposite extreme in feature sizes of PAA templates are those created from phosphoric acid as outlined in the next section.

### 3.3.4 Phosphoric acid alumina templates

Phosphoric acid is able to create features in a self ordering regime of 195 V at 0.3 M, the feature sizes in this regime are around 400–500 nm, which are no longer small relative to the wavelength of visible light so are not suitable for the intended application. By adjusting the acid concentration at a given voltage the feature size may be sensitively adjusted within voltage dependent limits.

#### 3.3.4.1 Phosphoric acid 100 V templates

The voltage of 100 V is not commonly used since it is not a reported self-ordering voltage. Since the aluminium films were relatively thin, the results from using self-ordering and non-self-ordering voltages become more similar, provided the concentration is well optimised. Doubling the concentration at 100 V from 0.25 M to 0.50 M gives a factor
of two hundred increase in steady state current density showing great flexibility in the possible parameters which can be easily accessed. The current density trend becomes less dominated by initial current decline and increasingly close to the standard current density trend with increasing concentration. The noise in the trend decreases with increasing current density and is likely caused by thin oxide layers produced during sequential evaporation of individual aluminium pellets. The interruption in the deposition process and allowing an appreciable oxide layer to form.

![Graph of current density versus time using \( \text{H}_3\text{PO}_4 \) (0.25 M, 0.41 M and 0.5 M) at 100 V and 2 °C. 0.5 M has ten times the current density of 0.41 M which has three times the current density of 0.25 M. The most structured trend is that of 0.5 M, the high current density produces large pores close together. The SEM of the alumina template is shown in figure 3.11.

Figure 3.10: Semi-log graph of current density versus time using \( \text{H}_3\text{PO}_4 \) (0.25 M, 0.41 M and 0.5 M) at 100 V and 2 °C. 0.5 M has ten times the current density of 0.41 M which has three times the current density of 0.25 M. The most structured trend is that of 0.5 M, the high current density produces large pores close together. The SEM of the alumina template is shown in figure 3.11.

SEM images of the structures shown relatively good organisation throughout the concentration range, for a non-ordered anodising voltage. The most order was seen at 0.25 M and many defects present at 0.41 M and 0.5 M which cause small pores to form between larger ones disrupting the structure. At 0.5 M the structures become very close together so would be unlikely to allow much light to pass through once filled with metal. The structure size is important because the light interactions become stronger with individual particles closer to half the incident light wavelength. Metal nanowire structures as large as 200 nm have yet to be studied as part of ordered array so the ability to fabricate the templates for those structures is a great advantage.

Intermediate PAA feature sizes between those produced by anodisation with phosphoric acid 100–500 nm and sulfuric acid 15–80 nm are produced using oxalic acid. The structure
3.3. RESULTS AND DISCUSSION

Figure 3.11: SEM image of an alumina template made using $\text{H}_3\text{PO}_4$ (0.25 M, 0.41 M and 0.50 M) at 100 V and 2 °C. The pores produced were; 60–160 nm (105 nm), 70–120 nm (86 nm) and 190–245 nm (215 nm) for 0.25 M, 0.41 M and 0.50 M respectively. For all images the scale bar is 1000 nm, shown red. Noise reduction was applied. The anodisation current density curves are shown in figure figure 3.10.

of which, are 30–80 nm as outlined in the next section.

3.3.5 Oxalic acid alumina templates

3.3.5.1 Single stage anodisation

Oxalic acid self ordering PAA is well established and produces the best results at 40V with a concentration of 0.3 M at between 0–10 °C producing 50 nm features. The current density versus time is relatively smooth and indicates a lack of oxide layers throughout the film thickness. The final decrease is due to the depletion of the available aluminium layer.

Figure 3.12: Current density versus time using $\text{C}_2\text{O}_4\text{H}_2$ (0.3 M) at 40 V and 1 °C, done in one stage of 10 minutes. The aluminium film thickness has been exhausted at 10 minutes, the current drops as the whole anodised area reaches the glass substrate. The SEM of the alumina template is shown in figure 3.13.

The PAA produced is relatively organised considering the anodisation was only a single stage, with pores formed with diameters 27–56 nm averaging at 47 nm. Ideally a
more narrow size distribution would be obtained but often very long anodisation times and aluminium thickness are required to enable this.

Figure 3.13: SEM image of an alumina template made using $\text{C}_2\text{O}_4\text{H}_2$ (0.3 M) a at 40 V and 1 °C. The pores produced were; 27–56 nm (47 nm). Each image has the scale bar displayed below. Noise reduction was applied. The anodisation current density curve is shown in figure 3.12.

A two stage anodisation trial was conducted, and a SEM for the result is depicted in figure 3.14. The aim of the trial was to determine the best ordered structure that could be obtained. The ordered domains have grown significantly with a second stage and the number of defects has been greatly reduced. However, some defects still remain and are concentrated around the metal’s grain boundaries, giving a clustered appearance.

Figure 3.14: SEM image of an alumina template made using $\text{C}_2\text{O}_4\text{H}_2$ (0.3 M) a at 40 V and 7 °C. The pores produced were 55–62 nm (58 nm).
3.4 Conclusions and outlook

Sputtered films were found to be far too impure to be usable for anodisation, the most likely cause of contamination appears to be from oxidation. The slow coating rate allowed a significant percentage of the template to be affected producing structural defects, which increased in severity with additional anodisation steps. That is in contrast to the literature[14], which report multiple stage anodisations to give an improved structure.

Evaporated metal films using large evaporation sources and high current was able to produce thick aluminium films at a deposition rate ten times the rate of sputtering. The purity as a result was significantly improved anodisation proceeded well with all acids attempted.

Using sulfuric acid and ethylene glycol ordered 17 nm PAA templates have been produced, to test the limit of the smallest structures possible to create using a PAA template. Some regions of the template appear greatly affected by the 2–5 nm surface roughness, which disrupted the structure. Because of the small scale of the sulfuric acid PAA they were difficult to resolve and an AFM was required. The AFM tip is not able to penetrate far into the pores making it difficult to test for the completeness of pore-filling in the metal plating stage.

Oxalic acid templates are resolvable by the majority of SEM and the atomic number contrast allows successful plating to assessed more easily. The PAA structure from a two stage anodisation was easily the most ordered from among those studied. They are also highly studied for this reason reducing the impact of any work done using them.

Phosphoric acid PAA templates created at low anodisation voltages such as 100 V, access a regime of pore sizes 80–200 nm at periods between 150–300 nm. For an incident wavelength these structures would be at the large-size limit for SP coupling and in a regime, which has been ignored in meta-materials research. The ordering is not very strong in this region, but ordered structures such as gratings are known to diffract, which would limit their use as an optical lens.


CHAPTER FOUR

METAL DEPOSITION AND PROCESSING
4.1 Introduction

Silver NW arrays are used in SERS detection[1] and in optical metamaterials such as hyperbolic metamaterials[2] and have been reported to enhance fluorescence[3]. The methods to create NW structures are developed in this chapter and are concerning the deposition of silver metal into a PAA template to create the NWs.

The PAA templates form a two dimensional hexagonal close-packed array of pores, metal plating inside this and subsequent removal of the template produces the negative of the PAA structure. Nanowires produced in this way inherit the properties of PAA templates, such as the high aspect ratios, small repeat period and long range ordering. This is all achieved without utilising expensive lithographic patterning techniques. The processes involved in metal plating are now discussed.

4.2 Background to metal plating

Electrolysis is a process of passing an electric current through a conductive solution, containing ions, termed the electrolyte. The electric current drives reactions at the electrodes making a circuit. Electroplating is a form of electrolysis, where the goal is to plate a metal, usually in the form of ions onto the cathode in the cell. There are two types of reaction inside a cell, oxidation and reduction, which are the loss and gaining of electrons respectively. These reactions occur at the cathode and anode, and are given by equations 4.2.1 and 4.2.2, respectively.

\[ M^{n+} + ne^- \rightarrow M \]  
\[ M \rightarrow M^{n+} + ne^- \]

The potential difference between two electrodes in an electrolytic cell, \( E \) is the potential difference between the cathode and the anode. The standard electrode potential, \( E^\circ \) allows comparisons between electrodes, the standard anode is the hydrogen electrode. Larger positive \( E^\circ \) values indicate the metal is a stronger oxidiser, more negative values indicate a stronger reducing agent. The standard electrode potential of several typically plated metals
4.2. BACKGROUND TO METAL PLATING

Table 4.1: Table of standard electrode potentials \( E^\circ \), of typical metals used in plating. Larger positive \( E^\circ \) values indicate the metal is a stronger oxidiser, more negative values indicate more a reducing character. Data from[4–6].

<table>
<thead>
<tr>
<th>Electrode</th>
<th>( E^\circ ) (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Ni}^{2+}(aq) + 2 \text{e}^- \rightarrow \text{Ni}(s) )</td>
<td>-0.23</td>
</tr>
<tr>
<td>( \text{Sn}^{2+}(aq) + 2 \text{e}^- \rightarrow \text{Sn}(s) )</td>
<td>-0.14</td>
</tr>
<tr>
<td>( \text{Cu}^{2+}(aq) + 2 \text{e}^- \rightarrow \text{Cu}(s) )</td>
<td>0.34</td>
</tr>
<tr>
<td>( \text{Ag}^+(aq) + \text{e}^- \rightarrow \text{Ag}(s) )</td>
<td>0.80</td>
</tr>
</tbody>
</table>

is given in table 4.1 below.

Ionic solutions frequently deviate from the ideal, non-interacting behaviour. Such non-ideal solutions, are no longer well described by just the concentration of ions, per unit volume. Since this does not quantify the availability, or interactions between the ions inside the solution. Thermodynamic activity \( \alpha \), is related to concentration by the activity coefficient \( \gamma \), which indicates the strength of these interactions for an ion. The measured potential \( E \) of an electrode is a function of the activity \( \alpha \), standard electrode potential \( E^\circ \), temperature \( T \) and the charge of the ion \( n \). The relationship is described by the Nernst equation[7] 4.2.3 below,

\[
E = E^\circ + \frac{RT}{nF} \ln \alpha, \quad \text{for} \quad (M^{n+}).
\]

where \( R \) is the gas constant and \( F \) is the Faraday constant, equal to 96485 C mol\(^{-1}\). In practical applications the molarity is often substituted for the activity, since the activity is not tabulated for all ion combinations. There is still practical insight to be found from such an approximation, but the results may only be interpreted qualitatively.

4.2.1 Metal plating techniques

Metal plating can be achieved by electroplating and electroless plating[8, 9]. Electroplating uses external electrical energy, applied to a pair of electrodes immersed in a conducting electrolyte to drive the plating process, this is illustrated in figure 4.1. The positive electrode is the source metal to be plated, the work-piece where the plating is applied forms the negative electrode. At the positive terminal electrical energy drives dissolution of positive metal ions into solution. At the negative terminal the electrical
energy drives reduction of positive metal ions, from the electrolyte solution. Since metal ions are reduced in close proximity to the work-piece surface, they form metallic bonds with the surface, and a coating of the new metal is formed. This process requires that the work-piece is a good conductor, since current flow is proportional to the plating rate.

Electroless plating uses chemical reactions with reducing agents to reduce the metal ions in solution to form solid metal nanoparticles, as is illustrated by figure 4.1. These nanoparticles form an auto-catalytic growth nucleus, and once large enough begin to precipitate, coating all of the solid surfaces in the solution. The initially seeded nanoparticles on the surface, catalyse further reduction of metal ions, and accelerate the deposition process. The reaction is limited by the concentration of metal ions and reducing agent.

Figure 4.1: Schematic of the silver electroless and electroplating process. In electroless plating reducing agents in solution, denoted "red", transfer electrons to metal ions to form metal nanoparticles, which deposit on all solid surfaces. In electroplating electrical current reduces silver ions at the work piece, forming a plated metal layer, specifically at the work piece.

Both methods require that the work-piece forms strong bonds to the surface, to ensure sufficient adhesion between the layers. Adhesion is affected by the compatibility of the crystal structures of the plated metal and the work-piece. Dissimilar materials are therefore less likely to form strong bonds, and rely more on non-specific interactions such as van der Waals forces. This sensitivity of the surface requires high standards of cleanliness, in order
to create a strong bond.

Unintended deposition may occur between metal ions and any solid surface, provided that surface is higher in the reactivity series, than the metal ion. For instance when copper is exposed to silver ions, the surface layer of copper is oxidised and enters the solution. The silver ions which were reduced in the process then coat the surface surface. This is termed immersion plating and is generally thin and highly fragile. The immersion plated films are thin, because of the limited diffusion of the metal ions into the solid surface.

4.3 Experimental

4.3.1 Silver electroplating background

A silver cyanide complex system is used commercially to electroplate silver. Additional components are also generally included to limit the reactivity of the surface, and prevent immersion plating and to control the rate of plating. These additives are called "brighteners" and they reduce the grain size of the metal film, and increase the film reflectivity. The hardness and film durability is also increased by including brighteners.

Cyanide is highly toxic and expensive to dispose of, so alternatives have been sought, modern non-cyanide plating systems utilise dimethylhydantoin or succinimide silver salts. Brighteners are also added to these systems, sulfur containing and other surface active compounds are often used to inhibit immersion plating.

Nicotinamide or polyetheneimine is added to control the availability of the silver ions in the dimethylhydantoin or succinamide complex, respectively. The effect of these additives is to disrupt and randomise crystal growth to reduce film roughness, and change the grain structure of the metal. Some example formulations are given in table 4.2.

4.3.2 Silver electroplating into PAA templates

A indium tin oxide layer was coated onto a glass slide by RF sputtering. A 99.99 wt% pure indium tin oxide target was used, sputtering was conducted with pure argon gas at a pressure of $1 \times 10^{-3}$ mbar, for a duration of 10–15 minutes at 10–15 W RF power. The sputtering power was the minimum permitted, as recommended in the literature, and the
Table 4.2: Table of typical silver electroplating components, for succinamide, dimethylhydantoin and cyanide based silver complexes. Data from

<table>
<thead>
<tr>
<th>Chemical component</th>
<th>Concentration (mol dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Succinamide based</strong></td>
<td></td>
</tr>
<tr>
<td>Silver mesilate</td>
<td>0.45</td>
</tr>
<tr>
<td>Succinamide</td>
<td>1.50</td>
</tr>
<tr>
<td>Boric acid</td>
<td>0.50</td>
</tr>
<tr>
<td>polyetheneimine</td>
<td>0.5 g/L</td>
</tr>
<tr>
<td><strong>Cyanide based</strong></td>
<td></td>
</tr>
<tr>
<td>Silver cyanide</td>
<td>0.37</td>
</tr>
<tr>
<td>Potassium cyanide</td>
<td>1.80</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>0.25</td>
</tr>
<tr>
<td>Commercial brightener</td>
<td>30 ml/L</td>
</tr>
</tbody>
</table>

Sputtering target-sample distance was maximised, to achieve greater uniformity.

Surface treating indium tin oxide is required prior to plating, to allow silver to bond to the surface. This is accomplished by mixing 99.99 wt% concentrated ethanolamine solution with potassium carbonate until saturated. This increases the pH and increases the de-protonation of the alcohol to give the alkoxide form. The liquid is decanted leaving the un-dissolved carbonate behind, and the indium tin oxide substrate is inserted for between 30–300 seconds, followed by rinsing in deionised water and ethanol and dried under nitrogen.

A protective titanium dioxide layer is added by sputtering. Achieved using a pre-oxidised titanium target, to form a layer of titanium dioxide on the surface. The titanium target is oxidised using an RF plasma comprising 40 % oxygen and 60 % argon, at a total pressure of $1 \times 10^{-3}$ mbar, with a power of 100 W, for 30 minutes. Titanium dioxide sputtering is conducted at 20–40 % oxygen with the remainder argon at a total pressure of $1 \times 10^{-3}$ mbar and a power of 100 W, for 5–8 minutes depending on the thickness required. The estimated sputtering rate is 1 nm/minute, quite low because of the low sputtering efficiency and large sputtering target-sample distance.

Acid etching to allow access to the base of the pore is accomplished by immersion of the PAA for 10–35 minutes in 5 %wt phosphoric acid. The etching time depends on the barrier layer thickness, which is a function of anodisation voltage and electrolyte, refer to section 3.1 for further details. Silver plating is achieved by inserting the sample into an
4.3 EXPERIMENTAL

electrochemical cell, and connecting the electrical contacts onto the top of the substrate, onto the conductive film. The plating solution of choice is poured into the cell and the silver anode is added. The voltage is then applied and the current is measured as a function of time.

4.3.3 Silver electroless plating background

Tollens reagent is a diammine silver(i) complex, which acts as a mild oxidising agent[10]. In the presence of the relatively reactive aldehyde group, the silver(i) is reduced to silver metal, and the aldehyde is oxidised to the corresponding carboxylic acid[10]. Glucose is in equilibrium with a non-cyclic aldehyde form and is often used in this process[11].

Early telescope mirror makers discovered that the plating process was improved by boiling the glucose in dilute nitric acid, in the Brashear process[11]. When boiled with nitric acid, glucose produces a complex mixture of products, the primary product, is the partially oxidised product, glucaric acid[12]. Glucaric acid is a $\alpha$-hydroxy carboxylic acid, and may be further oxidised by oxidative decarboxylation[12]. This produces a mixture of pentaric, tartaric, tartronic and oxalic acids[12]. Oxidation of the $\alpha$-hydroxyl group is also possible to generate 2-keto-gluconic acid[12]. Oxidation at the C$\textsuperscript{5}$ position is also possible to give 5-keto-gluconic acid[12, 13]. It was shown however that the reaction is not complete and some glucose will remain in the mixture, permitting reaction with the silver complex.

Silver and copper are in the same group in the periodic table, so are expected to share similar chemical properties, and both can be plated effectively. Electroplating copper is improved by addition of $\alpha$-hydroxy carboxylic acids, such as citric and tartaric acid as well as oxalic acid[14]. In electroless silver plating, the addition of tartaric acid is also known to improve the result[11]. These additions increase the range of the acceptable plating currents, by stabilising the solution[14], and increase the brightness of the deposit[14]. Glucaric and the other $\alpha$-hydroxy carboxylic acids also function as a metal complexation agents, so compete with the active diamminesilver(i) complex. It has been shown from electroplating experiments [15] that complexation agents in competition,
may work together synergistically to increase cathodic polarization. For electroless plating this makes the reduction process more energetically favourable, since the solution reducing agents are the "cathode" in that process. This follows because the \(\alpha\)-hydroxy carboxylic acids are also known reducing agents, and may reduce silver ions directly, or once a complex has formed[16].

### 4.3.4 Electroless silver plating in PAA

In order to electroplate a PAA sample, any air inside the pores was removed by sonication in deionised water for 30 seconds[17]. Then the PAA pores were widened in 5 wt% phosphoric acid for 30 minutes prior to plating, to access the substrate below from inside the pores. Two solutions were then prepared, a silver solution and a reducing solution.

The reducing solution contained 0.6 M glucose and 0.2 mM nitric acid, boiling this solution drove the oxidation of glucose, giving a mixture of products as detailed in section 4.3.3. The reducing solution was then cooled to room temperature using an ice bath prior to use, in order to control the reaction rate.

The silver solution contained 0.1 M silver nitrate. To this solution was added drop-wise, concentrated ammonium hydroxide solution containing 30% ammonia. Upon addition, black silver oxide was formed and subsequent additions of ammonium hydroxide dissolved this turning the solution clear. This formed the oxidising diammine silver(i) complex, \((\text{Ag(NH}_3)_2\text{)}^+\). To improve the plating rate the pH was lowered to 12 by adding potassium hydroxide, to a solution concentration of 0.23 M. This re-precipitated silver oxide, and then the minimum amount of concentrated ammonium hydroxide was added to reform the diammine silver(i) complex.

The silver solution and the reducing solution were then mixed, and the chemical transfer of electrons between the diammine silver(i) complex, and the reducing agents produced silver metal atoms. These atoms then seeded onto the surfaces inside the solution, forming a autocatalytic seed for further electroless plating. This resulted in the solution turning from colourless to brown upon the formation of these particles. The solution reaction then accelerated, turning black and then eventually dark brown, while the plating reaction...
4.3. EXPERIMENTAL

proceeded to coat the surfaces.

The electroplating solution plates every solid surface inside the solution, for optical devices silver plating is only desired inside the PAA and undesired on the reverse of the substrate. To ensure only the area containing PAA on the sample was silver plated, the anodisation cell was utilised, as depicted in section 3.4.

Once the pores were filled, plating was allowed to continue to ensure all pores were full. This formed a thick silver deposit completely covering the PAA. To remove this diamond paste (0.250 µm) manual polishing on a static polishing pad was conducted. Regular rinsing in deionised water was important to remove debris, to avoid it affecting the fragile structure.

Once the NWs were exposed, the pillar height could optionally be controlled by selective silver etching inside the PAA template. Neutral etchants were required, to avoid etching the PAA and ensure etching was only occurring vertically in the pores. A mixture of 0.3 sodium thiosulfate, 0.3 M potassium ferrocyanide and 3 mM potassium ferricyanide formed the basis of the etchant. Etching for between 5–8 minutes reduced the NW height and lowered the device’s optical absorption. This was adapted from Farmer’s reducer, a mild etchant used to reduce the density of silver on photographic negatives[18].

Once the NWs were at the correct height the PAA was etched selectively by concentrated 0.5 M potassium hydroxide solution, for a period of 5–10 minutes. This produced vertically aligned, organised silver pillars on a glass slide substrate with a controllable height and diameter.
### 4.4 Results and discussion

#### 4.4.1 Electroplating silver onto indium tin oxide

Electroplating inside a PAA template and form vertically aligned NWs, the bottom of the PAA’s pores must be opened by acid etching.

Electroplating requires an electrical contact, metals are often used, however when a metal layer is highly conductive, it also lacks transparency. In order to create a transparent device reliably, a transparent conductor is therefore required. Indium tin oxide is a common transparent tin-doped semiconductor, with a bandgap of 3.64 eV, corresponding to the UV wavelength of 341 nm.

#### 4.4.1.1 Sputtered ITO

![AFM of ITO surface quality](image)

Figure 4.2: AFM of ITO surface quality at different length scales, with a low RMS roughness of 0.63 nm.

The resistivity of 3.5–10 $\Omega$m is an order of magnitude higher compared to the literature, where $1.28 \times 10^{-4}$ $\Omega$m has been reported[19]. The sputtering system was not able to produce films of high purity, as was discussed in section 3.3.1, so it likely the films were contaminated during the sputtering process. This contamination is likely to have caused the large decrease in conductivity, attempts to renovate and clean the system did not improve the results.

Fortunately the high resistivity of 3.5–10 $\Omega$m in the indium tin oxide produced did not prevent electroplating, since the electroplating solution was designed to use only 1 mA or less of current. A plating voltage of 10 V or less sufficient to commence plating. More
important for the application of plating inside the PAA template was the low roughness of 0.63 nm RMS. Low roughness is a requirement of well ordered PAA, and any roughness below the aluminium layer will be transferred into the top surface of the aluminium film.

When direct electroplating was attempted the silver film formed, but floated free from the substrate upon termination of the process, evidence of very poor adhesion. Indium tin oxide is not able to form bonds with the plated silver directly, since they are not chemically compatible. Only the weak, non-specific physical forces, such as van der Waals forces were active. The ITO surface therefore must be treated, to increase the adhesion of plated silver, as ultimately stable high aspect-ratio structures are desired.

### 4.4.1.2 ITO surface treatments

To treat the surface ethanolamine was used since it is bi-functional, and in basic solution it forms an alkoxide $\text{NH}_2\text{CH}_2\text{CH}_2\text{O}^-$ ion. These ions rapidly attack the surface of indium tin oxide, and are chemically compatible with the alkoxide ion forming a strong bond. The amine $\text{NH}_2$ group is known to form strong chemical bonds to silver, thus allowing silver plating on a transparent conductor. This is the first time this method has been employed, and that chemically bonded silver films were formed on indium tin oxide by DC electroplating.

The method is extremely simple and applicable to a wide range of metal oxides, improving the hydrophilic and adhesion properties. This has applications in the biological sciences, to create electrical capacitive sensors on a transparent conductors, sensitive to protein binding to the exposed amine group could be possible. Indium tin oxide is also SP active in the near infra-red region around 1600–1900 nm, a shift in the SP resonance wavelength in a indium tin oxide could be used to detect biological molecules.

![Figure 4.3: Comparison of ITO surface wetting by water before and after immersion of room temperature MEA. Contact angles; 102°, 27°, 16°, for before, after 30 seconds and after 300 seconds in 100% MEA, respectively.](image)
This method was able to greatly increase the hydrophilicity of the surface after only 30 seconds in the treatment solution, with diminishing returns up to 300 seconds. The simplicity of the chemistry allows a speculative mechanism for the transformation to be proposed as in figure 4.4.

![Figure 4.4: Schematic showing a possible mechanism for the increase in surface energy. Since there are many such groups it could form an ordered surface array of hydrogen bonding acceptors which can effectively disrupt the structure of water. The newly attached amine can complex silver, the surface was previously unable and adhesion was poor.]

Silver plating onto the surface treated indium tin oxide was successful, and adhesion was ideal through the scotch tape test. In this test the powerful adhesive of the tape, was unable to remove the silver layer from the indium tin oxide layer. The most promising method in the literature for plating indium tin oxide by contrast, was only a partial success under the same conditions[20]. The same study also only marginally increased the contact angle with deionised water by 6.3°[20] and slightly decreased it with the silver plating electrolyte by 13°[20]. This minor increase in hydrophillicity is likely have lead to the lower interaction strength with the surface.

This is likely to have been caused by a weaker bond to the indium tin oxide, they utilised a silane surface active compound, 3-mercaptopropyl-trimethoxysilane, which is able to directly bond to the oxide surface. Ethanolamine is almost three times smaller than the silane they used, in terms of the number of atoms, it so would be expected to have a much higher surface density of silver bonding sites.

Unfortunately although indium tin oxide can be electroplated, to create silver NWs a PAA template needs to be made by anodisation of a layer of aluminium above the indium...
tin oxide. The highly acidic conditions and high applied potential, lead to the corrosion and detachment of the indium tin oxide layer. However, the anodisation conditions could be moderated using a chemically resistant transparent layer, such as titanium dioxide.

4.4.1.3 Titanium dioxide protective layer

Titanium oxide successfully protected indium tin oxide against corrosion in the anodisation process. It did however increase the resistivity to 20 Ωm, a 100% increase but acceptable for plating, just with increased applied potential. An AFM image of a sputtered titanium layers produced is shown in figure 4.5.

Figure 4.5: An AFM image showing how the concentration of O₂ affects the quality of the sputtered TiO₂, the RMS roughness was 0.77 nm and 1.16 nm for the 20% and 40% O₂, respectively. Percentage gas composition is the percentage of sputtering gas pressure, where the remainder is argon.

Critically for the creation of PAA templates upon this surface, the surface roughness of the applied titanium dioxide was low at 0.77 and 1.16 nm, when sputtered with 20% and 40% oxygen plasma content, respectively. The lowest average roughness was produced at 20% oxygen concentration, particles seen on the surface are likely to be atmospheric dust and not related to the film quality.
4.4.1.4 Electroplating alumina templates

Figure 4.6: A schematic of the desired end-product, (after removal of the PAA template) with different layer configurations trialled during electroplating experiments. **Left:** the silver nanowires are attached directly to indium tin oxide (ITO) which is coating a glass substrate. This configuration suffered from acid damage during anodisation. **Right:** A protective layer of TiO$_2$ has been inserted between the ITO and the silver to protect the ITO during anodisation.

The desired end product is illustrated in figure 4.6 the initial approach shown **left** was vulnerable to acid damage of the indium tin oxide during anodisation, a protective titanium dioxide layer was then added, shown **right**. The plating rate inside each pore of the PAA template is a function of the pore cross-sectional area. This is because the circuit current is related to the electrolyte-surface, contact surface area. Thus any variation in the pore diameter throughout the PAA template leads to a drastically different NW growth rates and uneven NW length[1, 21].

Figure 4.7: Electroplating silver on indium tin oxide electrode, protected by titanium dioxide protective layer. As compared to a control, plating silver onto copper, using SPA silver plating solution. In both curves there is an initial current spike from the rapid change in potential, which then settles as plating begins. Current density in the PAA pores increases with time, as the plating rate is less controlled by diffusion into the 30 nm PAA pores. Silver plating onto copper reaches a steady state after initially rough features become plated.
Plating was trialled using the commercial SPA silver tank plating solution, based on a silver succinamide complex. An outline of the compositions is previously outlined in section 4.3.1. The current density-time graphs for plating onto titanium dioxide protected, indium tin oxide inside a PAA template and a plating onto a flat copper plate as a control are displayed in figure 4.7.

There is a large difference in current density between the control and plating inside the PAA pores in figure 4.7. The difference is likely caused by the reduced surface area for plating inside a PAA template, and the reduced diffusion of metal ions to the base of the PAA pores, compared to a planar substrate. The corresponding SEM image of the plated silver is shown in figure 4.8, many dark regions are present where NWs are missing forming defects in the structure. Neighbouring the dark regions are intensely white, larger structures where the plating current appears to have been flowing to preferentially. The most ordered section with the most consistent NW size on the left side of the figure, towards the centre, and interestingly it also has the fewest defects. The reduced defect count is therefore likely to be linked to the more consistent PAA pore size.

Figure 4.8: SEM image of silver NWs, electroplated at 20 V using SPA silver plating solution. The PAA template was anodised using 0.3 M oxalic acid, 40 V at 2 °C. The template was first widened (35 min 5 %wt H₃PO₄) to allow contact to the bottom electrode. The SEM shows silver nanowires shown white on a glass surface, which appears black. The PAA pore filling was quite poor and there are many defects, seen as dark spots. The NW grown rate was very uneven with some features more than a factor of four larger than their immediate neighbours. The scale bar is 500 nm. Noise reduction was applied.
4.4.2 Thermal deposition of silver

To attempt to improve the quality of the metal nanopillar arrays, a thermal synthesis method was trialled, which relies upon the high temperature instability of silver oxide, decomposing to silver metal. The equation for the decomposition is shown below,

\[
2 \text{AgNO}_3(\text{aq}) + \text{O}_2 \xrightarrow{450^\circ \text{C}} 2 \text{AgO(s)} + 2 \text{NO}_2(\text{g}) + \text{O}_2(\text{g}) \quad (4.4.1)
\]

\[
2 \text{AgO(s)} \xrightarrow{450^\circ \text{C}} 2 \text{Ag(s)} + \text{O}_2(\text{g}) \quad (4.4.2)
\]

This was originally investigated by[17] to create arrays of silver NWs in an alumina template. The regularity and structure of the films was not of primary concern to them, as their intended application was to create solution dissolved NWs[17].

Figure 4.9: SEM of the different scales of non-uniformity across a single thermally produced sample. A shows the larger debris, B and C show the metal structures but with large height variation, major structural defects and debris. The green scale bar is 10000 nm and the red scale bars are 200 nm.

Several problems were encountered while replicating their work, the amount unwanted debris was significant, as shown in figure 4.9 A and C. The defect density was very high
even in the most ordered areas of 4.9 B and C. The sample plating uniformity of the structure was very low, this is also demonstrated by 4.9 since all three sub-figures are from the same sample within a 50 $\mu$m$^2$ area.

### 4.4.3 Electroless silver plating

The desired end-product from the electroless silver plating on glass is shown in figure 4.10. Electroless silver plating has been used before to plate PAA templates[22], the application was to create silver NW particles in solution. No systematic study was conducted of the order of the structures produced[22]. In order to removed trapped air to allow the solution into the pores, they needed to keep the PAA under vacuum for 8 hours[22]. Reaction times for the process they quoted as 2 hours, with PAA etching times of 12 hours[22]. The structure they produced had over-grown the templates and had debris on the template surface[22], which would greatly disrupt it’s use in an optical application. These experimental details show their method is highly time-inefficient, and not suitable to produce optical-quality ordered NWs adhered onto a glass substrate.

In order to develop a practical method using electroless plating, there are several problems to solve, mainly the trapped air in the PAA, and the excess silver debris on top of the PAA template. Using a vacuum[22] is not a very time-efficient method of removing trapped air, studies have shown that applying ultrasonic can quickly achieve the same result in minutes[17]. It was shown that the overgrowth of silver on the PAA templates...
may be removed by polishing with an ultra-fine diamond paste[2], producing an optically flat surface finish[2].

Applying the aforementioned techniques to this research produced an electroless method, outlined in section 4.3.4, which is state of the art for the creation of ordered silver NW arrays. During tests the electroless solution traditionally used in mirror silvering by Brashear[11] was used as the starting formulation. The results from using the original Brashear formulation are illustrated in figure 4.11, the interior surface was found to have significant debris in addition to the desired silver film. The light was visible scattered by the debris, indicating their diameter was 150 nm or above and the yellow colour is characteristic of the SP resonance of large silver nanoparticles[23, 24]. This indicates that there it that there are larger nanoparticles of silver, which have precipitated from solution. This precipitation of large particles disrupted the desired autocatalytic plating process of individual metal ions from solution.

![Brashear process](image1.jpg) ![Experiment 6](image2.jpg)

Figure 4.11: Photographic comparison between the traditional Brashear process and the newly developed procedure shown in table 4.3. To achieve a good optical quality the original author Brashear claimed, the film must be "rubbed vigorously with a pad of clean absorbent cotton"[11], this is not required with the process in experiment 6.

The discussion of the contents of the plating solution in section 4.3.3, showed that a mixture of many silver complexation agents were produced by boiling glucose with nitric acid. These complexation agents are able to coat the surface of larger nanoparticles in solution, increasing their solubility and helping to hold them in suspension, preventing precipitation. The higher the concentration of nitric acid treated sugar in the plating solution, the better the plating should become. The film produced is indeed improved with higher concentrations of nitric acid treated sugar, as shown by table 4.3, but the plating rate
4.4. RESULTS AND DISCUSSION

suffers significantly the more that is added. The best compromise was found in experiment 6, the parameters are shown in table 4.3 and the plating result is shown in figure 4.11.

Table 4.3: Table comparing the traditional Brashaver process to the newly optimised electroless silver plating process. A subjective scale (1–5) is used to compare the different experiments. **Adhesion** subjective scale; (1–2) film disturbed by a light touch, (3–4) unaffected by polishing cloth, 5 hard to remove with sharp implement. **Reflectivity** subjective scale; (1–2) reflection barely visible and surface visibly rough from particles 10000 nm or more, (3–4) reflection is clear but some scattering is present from particles 1000 nm or less, 5 reflection is perfect so particles are smaller than the light’s wavelength. **Colouring** subjective scale; (1–2) serious discolouring showing plating solution highly unstable, (3–4) small amount of discolouration visible, 5 no apparent discolouration. **Film thickness** subjective scale; (1–2) film barely visible, (3–4) film is obvious and around 40 nm thick but light visible through, 5 film is optically thick and >50 nm. Film colouring from organic contamination from an unstable plating solution is related to the film adhesion.

<table>
<thead>
<tr>
<th></th>
<th>Brashear</th>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Exp 3</th>
<th>Exp 4</th>
<th>Exp 5</th>
<th>Exp 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (Mol dm(^{-3}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric acid</td>
<td>0.0605</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.0002</td>
</tr>
<tr>
<td>KOH</td>
<td>1.8897</td>
<td>0.2376</td>
<td>0.2376</td>
<td>0.2376</td>
<td>0.2376</td>
<td>0.2376</td>
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</tr>
<tr>
<td>Ethanol</td>
<td>2.542</td>
<td>0.0000</td>
<td>3.4229</td>
<td>3.4229</td>
<td>3.4229</td>
<td>3.4229</td>
<td>0.0000</td>
</tr>
<tr>
<td>AgNO(_3)</td>
<td>0.4906</td>
<td>0.1001</td>
<td>0.1001</td>
<td>0.1001</td>
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<td>0.1001</td>
</tr>
<tr>
<td><strong>Adhesion</strong> (1–5)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Reflectivity</strong> (1–5)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Colour</strong> (1–5)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Film thickness</strong> (1–5)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Glucose : AgNO(_3) ratio</td>
<td>0.8637</td>
<td>1.664</td>
<td>3.6977</td>
<td>7.987</td>
<td>19.9674</td>
<td>9.9837</td>
<td>5.9902</td>
</tr>
</tbody>
</table>

This high quality electroless silver plating was then applied to PAA, the over-growth of silver was countered by polishing with ultra-fine diamond paste. Figure 4.12 A illustrates the structures revealed by polishing, while inside the PAA template to give a structure as illustrated by figure 4.12 B. This is a large step forward, as every PAA pore is filled despite the non-ideal hexagonal arrangement. Obtaining the ideal PAA structure is difficult, can be over small areas, and often expensive in thin film applications as was discussed in section 3.1. The straight NW profile is shown in figure 4.12 C, some structures were damaged by cleaving the sample, on the whole the NW profile is straight. The NWs are only 112 nm high and 64 nm in diameter approximately a 2:1 ratio, a smaller height helps to limit optical absorption losses as discussed in section 1.9.4.
Figure 4.12: SEM of metal NWs produced with a phosphoric alumina template produced at 100 V. Two different magnifications A–B show an overview with a scale bar of 2000 nm, and C is a higher magnification scale bar 500 nm. A–B show the ordering of the NWs is acceptable, A is a 500 V SEM showing the efficiency of surface polishing. In C the image is taken at 45 degrees and shows the vertical profile of the NWs. The NWs are 47–76 nm (64 nm) in diameter and 100–120 nm (112 nm) tall.

The same procedure was successfully adapted to the more unusual PAA system, produced by anodizing at 100 V with phosphoric acid, giving larger 190 nm diameter NWs, and is illustrated in figure 4.13. This will allow a comparison in properties between two very different size regimes.

Figure 4.13: SEM of metal NWs produced with a phosphoric alumina template produced at 100 V. Two different magnifications A is an overview with a scale bar of 3000 nm, and B is a higher magnification scale bar 1000 nm. A shows the ordering of the NWs is acceptable, from B the NWs are 150–260 nm (190 nm).
In the literature it is much more common to electroplate the silver pores\cite{1, 21}, typical literature results using this method to non-ideally structured PAA are displayed in figure 4.14 A and B. The defect density from missing features cannot compare to that of the electroless plating in this work. It has been predicted that defects from missing structures will create light scattering centres\cite{2}, making their presence unsuitable for optical devices.

Figure 4.14: Results from common literature methods employing metal layer deposition and electroplating into the PAA pores, illustrating the high defect densities present. Adapted from \cite{1, 21}.
4.5 Conclusions and outlook

All known methods of silver plating inside PAA templates were trialled, including electroplating, thermal deposition and electroless plating, which produced the most promising results. Thermal deposition and electroplating suffered from a high density of missing NWs, large amounts of debris and a lack of uniformity. The PAA pore filling with thermal deposition was particularly poor as little of original the structure was obvious.

Electroless by contrast had all of the PAA pores filled, regardless of amount of order in the PAA structure or the size of the pores. The Brashear process for electroless plating was optimised to produce this result. The chemical processes were not initially understood by Brashear, since it is highly complex and produces a mixture of products[12]. By understanding the reactions occurring, it allowed the complexation and reducing agents within the plating formulation to be identified for the first time. The concentration of these agents was then varied to study the effect on the film adhesion, reflectivity, film colouration and the plating rate. A strong relationship between the film adhesion, reflectivity and colour was identified. This suggested the presence of particles, which would disrupt the adhesion, reduce the reflectivity by scattering and the colour observed corresponds to the SP resonance of silver nanoparticles[23]. The improvements in the quality of electroless silver plating are significant and may have commercial relevance, as it required no surface activation and plated an entirely insulating surface.

Novel selective silver etching methods were developed, to etch the NWs inside the PAA template to allow control the NW height. This allows the optical losses in the device to be greatly reduced. This combined with the control of the NW diameter and period from adjusting anodisation conditions, allows previously not reported control over the device properties.


(14) J. E. Stareck, US patent 2250556, 1941.


CHAPTER FIVE

OPTICAL DEVICE PROPERTIES
5.1 Introduction

Super resolution microscopy has revolutionised the field bioimaging allowing unprecedented detail of fine structure of up to 40 nm[1]. For the impact of super resolution to be more widespread than from just a small number of specialist groups, an alternative approach to super resolution microscopy is needed.

The proposed alternative is uses a materials based approach, where a silver NW array on a microscope cover-slip slide achieves additional focusing[2–4]. This would preserve compatibility with existing equipment and techniques. In addition it would not require without sample modifications, only requiring that the sample be placed on a new type of cover-slip purchased as a consumable. The silver NW array on the microscope cover-slip would focus light using the intrinsic negative refractive index of the array[5, 6]. Negative refractive index metamaterials are discussed in the next, in section 5.1.1.

5.1.1 Negative refractive index metamaterials

Refractive index is a dimensionless quantity related to the density of a material, which describes the phase velocity \( v_p = \frac{\omega}{k} \) of light in that medium, compared to vacuum[7]. Typical transparent materials have a refractive index \( 1 < n < 4 \)[7]. The material refractive index \( n \), can be described by,

\[
 n = \frac{c}{v_p} \quad (5.1.1)
\]

where, \( c = \left( \sqrt{\varepsilon_0 \mu_0} \right)^{-1} \) \quad (5.1.2)

and, \( v_p = \left( \sqrt{\varepsilon \mu} \right)^{-1} \) \quad (5.1.3)

inserting gives, \( n = \pm \sqrt{\left( \frac{\varepsilon_t \mu_t}{\varepsilon_0 \mu_0} \right)} \) \quad (5.1.4)

where relative permeability \( \mu_t \) is the relative magnetisation of a media in an applied magnetic field compared to a vacuum, \( \mu_0 \)[7]. Typically for non-magnetic media this is \( \mu_t = 1 \). Relative permittivity \( \varepsilon_t \) is defined as the resistance to forming an electric field in a medium, compared to vacuum \( \varepsilon_0 \).
A negative value of $\epsilon_t$ or $\mu_t$ or both, implies an amplified local electromagnetic field is supported in that medium. This is consistent with the properties of SPs, for enhancing local electromagnetic fields see section 1.9.5. Negative refractive index materials are in several categories, but can have negative values for both $\epsilon_t$ and $\mu_t$[6]. In order to achieve negative refraction the phase velocity is required to be of the opposite sign, which requires taking the negative root of equation 5.1.4.

One of the major challenges with this class of material, is the absorption loss associated with their use can be substantial[6, 8]. This is especially true towards the blue region of the visible spectrum[9], this was demonstrated by a dramatic reduction in the quality factor around 400 nm in section 1.9.5 depicted in figure 1.19.

The first studies on negative refractive index materials, were conducted in the microwave[5, 10] region of the spectrum. These experiments used a flat "slab lens" consisting of an array of split-ring resonators[5, 11], which had a simultaneous magnetic and electric response $\mu_t = \epsilon_t = -1$. A schematic of the split-ring resonator is shown in figure 5.1

Figure 5.1: Schematic of a microwave split-ring resonator, made from two concentric broken circles of a conductor, such as copper. Each circle has capacitance and inductance across the gap and between the two concentric circles.

Obtaining negative $\epsilon_t$ and $\mu_t$ in a single bulk material at visible frequencies, has not been possible without patterning or structuring in layers[2, 3, 8, 12–15]. A negative $\epsilon_t$ and $\mu_t$ has been achieved using a layered fish-net structure, but only in the IR[16]. The onset of negative refraction is at 1450 nm, and at the peak of transmission of 14 % of the light was able to pass[16], good for a device of this class[8]. The design is of alternating layers of silver and magnesium fluoride. An ion beam miller patterned the trenches into the stack structure, which is illustrated in figure 5.2.
An alternative strategy is to rely on noble metals, with only negative $\epsilon_r$, and neglect the magnetic material response from $\mu_r$. The SP currents oscillate at high frequency, generating magnetic fields in neighbouring conductors[7]. One example was termed a hyperbolic metamaterial[4, 17–21] detailed next.

## 5.1.2 Hyperbolic metamaterials

Hyperbolic metamaterials (HMM) extend the range of spatial frequencies which can be transmitted compared to a conventional lens[4, 17, 20, 22–26]. High spatial frequencies are carried in evanescent fields and are amplified by the action of SPs in the structure. Two popular HMM structures are shown in figure 5.3, the SPs are restricted to either thin isolated surfaces stacked vertically or into vertically aligned narrow wires. Importantly the wire thickness and period or layer thickness are all sub-wavelength, so local SPs can be excited.
Figure 5.3: Schematic showing two popular HMM geometries, (A) Metal NW and (B) metal planar stack separated by dielectric spacers. Key features such as layer thickness or feature size are sub-wavelength to aid local SP coupling and electromagnetic response is direction dependent.

Since the SPs are restricted spatially there is a spatial disparity in the $\epsilon_r$ so we must consider each unique plane or axis separately.
5.1.2.1 Nanowire metamaterials

For a NW system shown in figure 5.3, the $\epsilon_i$ has the form [18, 19, 27],

$$\epsilon_{\text{nanowire (x,y)}} = \frac{\epsilon_d[(1 + p)\epsilon_m + (1 - p)\epsilon_d]}{(1 - p)\epsilon_m + (1 + p)\epsilon_d} \quad (5.1.5)$$

$$\epsilon_{\text{nanowire (z)}} = \epsilon_m + (1 - p)\epsilon_d \quad (5.1.6)$$

where $\epsilon_d$ and $\epsilon_m$ are the relative permittivities of the dielectric and the metal respectively. The fill factor $p$ is the fraction of the surface in the x-y plane covered by the NWs, as depicted in figure 5.4. The sign of $\epsilon_{\text{nanowire (z)}}$ is the opposite of $\epsilon_{\text{nanowire (x,y)}}$, giving the material a strong anisotropic field response.

![Figure 5.4: Applying equations 5.1.6 and 5.1.5 to common NW geometries for bare silver NWs ($\epsilon_m = -7.1$), immersed in water ($\epsilon_d = 1.33$), illuminated at 450 nm.](image)

Negative refraction has been shown in the NIR and visible spectrum as in figure 5.5[22]. In order to observe it, they had to use scanning near-field optical microscope, which directly probes the near-field around the structure with a sharply tapered optical fibre[28].
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Figure 5.5: Demonstration of negative refractive index with a non-magnetic material at visible frequencies, through a NW hyperbolic metamaterial. A shows the structure and how they took the measurement. (B–D) Applied Snell’s law is used to prove the negative refraction which is only present for the TM polarised light of either wavelength. Figure adapted from [22].

It is notable that they were not able to reduce the wavelength further than 660 nm. There is strong fluorescence emission[29] from the alumina templates when excited at wavelengths below 500 nm. This clear limitation is often neglected in the literature [22], the alumina template needs to removed after forming the NW arrays if they are to be excited close to the blue region of the visible spectrum.

Figure 5.6: **Left** Absorption spectrum of porous alumina template, **Right** Experimental fluorescence spectrum of a porous alumina template made using oxalic acid, the most commonly used. Absorption is non-zero at 500 nm and is readily excited throughout the visible. Adapted from [29].

If the optical losses can be controlled it has been predicted that negative refractive
index materials would be efficient focusing components[6]. The amplification properties of SPs allow the otherwise short-range evanescent fields to be transmitted to distanced far beyond a fraction of a wavelength[6, 15, 30]. Experimental verification of this is shown in figure 5.7. This was again shown using scanning near-field optical microscopy. It was not possible for these waves to be transported to the far-field of an imaging microscope objective[2]. It did however prove the principle, of evanescent amplification and transport achieved through the device, over a distance of more than six wavelengths.

Figure 5.7: A: is a schematic of the overall experiment to image through a NW based HMM. B: SEM image of the structure to be imaged optically. C: SNOM image of the "NEU" structure as seen through the HMM material, showing transport of evanescent waves a distance of six wavelengths. Evanescent waves contain the high frequency detail information, which normally decay significantly in amplitude after only a single wavelength. D: SNOM image of the structure as seen directly, and not through a HMM material. Figure adapted from [2].
5.1.2.2 Planar stack metamaterials

This is a planar stack of thin metal layers separated by dielectric spacers where the plasmon excitation is confined within each plane but may couple inductively between them. The permittivity of the medium can be approximated in each direction by,

\[
\epsilon_{x,y} = p\epsilon_m + (1 - p)\epsilon_d \tag{5.1.7}
\]

\[
\epsilon_z = \left(\frac{p}{\epsilon_m} + \frac{1 - p}{\epsilon_d}\right)^{-1} \tag{5.1.8}
\]

where, \( p = \frac{t_m}{t_m + t_d} \tag{5.1.9} \)

the volume filling ratio \( p \) for the dielectric-metal repeating unit, depends on the metal \( t_m \) and dielectric \( t_d \) layer thickness. This affects the medium’s transition from pure dielectric at \( p = 0 \) to metamaterial at intermediate values \( 0 < p < 1 \) between to pure metal at \( p = 1 \). An example showing variation in \( \epsilon \) with \( p \) in a planar stack metamaterial is shown in figure 5.8 for a stack of silver and titanium dioxide at 450 nm illumination.

![Figure 5.8: Applying equations 5.1.7 and 5.1.8 with varying volume filling factor p and using silver (\( \epsilon = -7.1 \)) and titanium dioxide (\( \epsilon = 1.68 \))[31], excited at 450 nm.](image)

Conventionally in order for the material response to be that of a HMM, the layer thickness must be small relative to the wavelength \( t < \frac{\lambda}{10} \), in order to excite local SPs[26]. This constraint also ensures that the light does not interact strongly with just one layer, but the structure as a whole[6]. The following condition must also be met in order for SPs to be excited in one axis only and generate the anisotropic optical response,
\[ \epsilon_{x,y} \epsilon_z < 0. \quad (5.1.10) \]

The loss associated with the structure originates primarily from resistive losses and from internal reflection at the interface though this can be minimised in this way,

\[ |\epsilon_d| \approx |\epsilon_m|. \quad (5.1.11) \]

There is also dispersion from the frequency dependence of \( \epsilon_d \) and \( \epsilon_m \), these can all be tailored for the application of interest and region of the spectrum used. The restrictions on the dielectrics used are suitable transparency, compatibility with manufacturing method and good matching as given by equation 5.1.11. Noble metals are often used in the visible and semiconducting metal oxides are used in the infrared.

### 5.1.3 Metamaterials as tools in microscopy

![Figure 5.9](image)

Figure 5.9: **Left** A schematic of the experiment with the chrome (Cr) mask producing sub-wavelength features which then illuminate a silver slab lens which amplifies the evanescent waves and transfers the structure into the photoresist (PR) shown in A, compared to the control without the silver B. Figure adapted from [3].

Evanescent waves have been shown to be transferable into a light sensitive imaging medium, such as photo resist using a simple silver slab lens shown in figure 5.9. The exposure was at 365 nm, and produced sub-wavelength features which were 60 nm in diameter on a 120 nm period[3], the features were \( \lambda/5.93 \). The control in figure 5.9 B,
without the silver slab lens, was unable to produce fine structure and gave a solid block, demonstrating the importance of evanescent waves transport from the mask into the photoresist by the thin silver layer.

Figure 5.10: A Schematic of the cylindrical multilayer stack "hyperlens", consisting of alternating layers of 35 nm thick silver and titanium dioxide and a patterned chrome (Cr) top-layer providing the image. B An example using the device with two patterned lines spaced 150 nm apart and resolving the image and not being able in the control. C Line profiles from the images given in B. D More complex imaging is also possible as shown but it is clear that the curved multilayer stack has position-dependent brightness with the central part illuminated more strongly for the same line-thickness. Figure adapted from [17].

The challenge is to then transport light from the near-field into the far-field where it may be imaged by a microscope objective. It was recognised that the planar geometry of the silver slab lens was unable to achieve this, so a HMM curved stack with convex geometry was made to take advantage of the negative refractive index and evanescent mode guiding[17], see figure 5.10. The stack was made from alternating layers of 35 nm thick silver and aluminium oxide. At first a cylindrical design was produced that resolved two sub-wavelength features only 150 nm apart, which is close to the 120 nm diffraction limit for 365 nm with the best optics. Clearly the control was unable to resolve the two features in such a challenging case and produced only a single feature proving the principle of far-field imaging. The image was magnified from 150 nm to 352 nm, a factor of 2.3, so the previously unresolved features were now resolvable.

In order to resolve 2D objects a three dimensional curved stack HMM was produced,
with 30 nm alternating layers of silver and titanium pentoxide (Ti$_3$O$_5$) and a fill factor of 1. This structure shown in figure 5.11 achieved 2.1x magnification. The original 180 nm separated, sub-wavelength features were magnified above the diffraction limit to 360 nm, so they could be resolved with 410 nm illumination by a microscope objective in the far-field. This device proved an important principle, demonstrating that it is possible to image arbitrary shapes beyond the diffraction limit. Without the complexity and cost of super-resolution microscopy, which is quite a barrier to their adoption. There are numerous shortcomings in to this work however; the images are of artificial, high contrast structures and only within the small area inside the lens can objects be imaged. This structure is also recessed compared to the surrounding substrate, making it difficult to utilise for general microscopy, and impractical for most samples.

Figure 5.11: Spherical curved stack with objects patterned into chrome on top shown in SEM (a, d) and optically through the lens (b,e). The corresponding line profiles are in (c, f) and a schematic of how the magnification of sub-wavelength features into the far field occurs is shown in g. Figure adapted from [4].

### 5.1.4 Fluorescence enhancement

HMMs have been used to increase chromophore emission rates $\Gamma$, by coupling to plasmon and amplified high-k modes. This shortens the chromophore fluorescence lifetime and increases the duty cycle of emission, though there are also material absorptions and non-radiative decay into the HMM which reduce the amplitude of the effect[25]. HMMs were compared to thin films with only SPs and were found to be more efficient at increasing radiative decay. The mechanism depends on the imaginary component of the reflection coefficient $\text{Imag}(r_p)$, the dipole moment perpendicular to the surface $\mu_{\text{perp}}$, and the distance to the device, $d$,.
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\[ \Gamma_{\text{high-k}} \approx \mu_{\text{perp}} \text{Im}(r_p) \frac{8\hbar d^3}{(5.1.12)} \]

where, \( \text{Im}(r_p) = \frac{2\sqrt{|\epsilon_{x,y}\epsilon_z|}}{1 + |\epsilon_{x,y}\epsilon_z|} \) \quad (5.1.13)

\[ \Gamma = \Gamma_{\text{vac}} + \Gamma_{\text{plasmon}} + \Gamma_{\text{high-k}} \quad (5.1.14) \]

where \( \Gamma_{\text{vac}} \) is the emission rate in a vacuum, \( \Gamma_{\text{plasmon}} \) is the emission rate due to plasmons, \( \Gamma_{\text{high-k}} \) is the emission rate due to high-k states.

Planar stacks decreased fluorescence lifetimes by a factor of 4 with CdSe/ZnS colloidal quantum dots [20]. Using a relatively inefficient IR-140 laser dye, fluorescence lifetimes were decreased by a factor of between 3.5–5.7, with corresponding emission enhancements of 27–49. The dye environment was relatively quenched inside a polymer matrix [23]. With a dye above NW array the fluorescence lifetime of the IR-140 laser dye, reduced from 760 ps to 125 ps, a factor of 6.1 shorter [24], which is illustrated in figure 5.12.

![Figure 5.12: Curves 1 to 3 are controls on porous alumina, gold and silver films and 4 is on the porous alumina when filled with silver NWs a HMM. Figure adapted from [24].](image)

The effect is likely to have been less obvious with an efficient dye, excited in a non-quenched environment and using a superior microscope objective; with a NA larger than 0.75 [32, 33]. The power used was <1 \( \mu \)W, which is very low and biases any small increases in efficiency not seen at higher powers.

The argument used by [23] to justify that \( \Gamma_{\text{high-k}} \neq \Gamma_{\text{plasmon}} \) is not due to plasmons by
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comparison to a single layer is a little misleading. Firstly it has been shown that SPs can transport high-k information[21]. Secondly it assumes that the plasmonic component should be the same between a single layer, and a multi-layer structure. In a multi-layer structure, each layer will support independent SPs, and there will be strong magnetic coupling between the SPs[8, 12, 13] of adjacent layers. This is because the distances between the layers are small compared to the wavelength, at 19 nm compared to 635 nm[25]. The magnetic coupling between SPs of different layers leads to the apparent amplification of the signal compared to a single layer.

Nanowires appear slightly more efficient at fluorescence lifetime quenching than planar stacks. The chromophore can also be allowed inside the structure if the alumina template is removed, which offers a greater interaction surface. On the exterior too, the hyperbolic dispersion appears to be stronger, resulting in greater lifetime reduction[24]. The $\Gamma_{\text{plasmon}}$ term was found to have an explicit dependence on the fluorescence lifetime and the laser intensity at the sample[34] with silver nanoparticles. The average lifetimes of a ruthenium complex varied from 12.6 ns to 1.3 ns at 0.03 $\mu$W and 32.60 $\mu$W respectively. Molecules inside gold nanoapertures had a consistent factor of ten higher brightness than in solution[35] depicted in figure 5.13. Using nanoapertures to confine light is discussed further in section 2.

![Figure 5.13: Count rate per molecule for a nanoaperture in a thin gold film, compared to open solution as a function of laser power. Figure adapted from [35].](image)
5.2 Experimental

5.2.1 TCSPC on the surface of the NW devices

TCSPC was measuring the fluorescence lifetime of 75 \( \text{nm} \) rhodamine 110 on the surface of the devices and in a solution control on glass. The power level was adjusted relatively low to 12 \( \mu \text{M} \), to try and reduce reflection as much as possible. Reflection from the diode laser at 450 nm was filtered using two 525/50 and a 500 nm long pass, emission filter. The devices were inverted so the excitation laser beam focused onto the solution facing side of the device. Excitation was conducted with a high power Olympus 40x water immersion objective, with a NA of 1.2. The experimental apparatus is described in detail in section 1.8.3.

5.2.2 Transmission fluorescence microscopy

Transmission fluorescence microscopy measures the fluorescence created from light transmitted through an object, exciting a fluorescent dye solution above. This is a powerful tool to compare the focusing of a device compared to the control without, as the fluorescence intensity distribution is related to the reflective properties of the object the light has passed through.

The experimental design is illustrated in figure 5.14. A CW diode laser (450 nm), is expanded by focusing into a 25 \( \mu \text{m} \) pinhole using a 20x objective, and then collimated using a 12 cm focal length planoconvex lens. The beam is then reflected onto the lens of an adapted Olympus Student’s monocular microscope. Where the beam is focused through the microscope cover-slip with a NW array structure on the top surface, immersed in a fluorescent dye solution. The emitted fluorescence is then collected by a second objective lens, and the light is then filtered to remove the excitation light and focused onto a Carl Zeiss Flektogon 35mm, attached to a Fujifilm X-E1 camera detector.
Figure 5.14: Schematic of the transmission fluorescence microscopy experimental setup. This is used to directly measure the refractive properties of an object transmitting light into a solution containing fluorescent dyes.
5.3 Results and discussion

5.3.1 Absorption spectra

5.3.1.1 Absorption spectra oxalic acid derived NW arrays

The absorption spectrum of NWs from oxalic acid templates are illustrated in figure 5.15. The absorption spectra of nanowires from oxalic acid templates have two peaks, a transverse absorption and a longitudinal absorption[36]. The absorption with and without the PAA template are 432 nm and 424 nm for the transverse absorption, and 467 nm and 453 nm for the longitudinal absorption, respectively. The thin film interference was removed for clarity in 5.15 right, using a low pass filter.

The absorption at wavelengths longer than the longitudinal peak (500–750 nm) is due to coupling between NWs[8, 12, 13, 37]. The resonances appear to be broadband outside of the PAA template, and clustered into two groups inside the PAA template at 520 nm and 700 nm. More localised coupling inside the PAA template could have lead to the quantisation into two groups. By coupling inside of the hexagonal close packed unit cell at 520 nm, and coupling between neighbouring unit cells at 700 nm. The broadband absorption outside of the PAA template suggests a wide range of coupling distances. This conclusion appears supported by the increase in absorption at 700 nm. This is from long distance NW–NW coupling, when the PAA template was removed. The reduction in the relative permittivity of the structure transitioning from alumina to air between the NWs, is 2.25[36] to 1, respectively. This reduction in $\varepsilon_r$ would allow longer distance NW–NW coupling, and lead to broadband absorption, which is not clustered into two groups.

In order to gain enough transmission for absorption characterisation, the nanowires were etched significantly inside the PAA to produce 120 nm tall nanowires. For the phosphoric produced NWs in section 5.3.1.2, this was not necessary as the starting absorption was significantly lower for high aspect ratio NWs.
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Figure 5.15: Absorption spectrum of metal NW arrays, produced using oxalic acid at 40 V without Left and with Right additional low pass signal processing. For Oxalic acid the transverse absorption peak inside the template shifts from 432 nm to 427 nm when the PAA is etched away. The longitudinal peak shifts from 467 nm inside the PAA to 453 nm when the PAA is etched away. Two broad NW–NW coupling peaks at 520 nm and 700 nm can be seen when inside the PAA template, separated by a dip in absorption.

5.3.1.2 Absorption spectra phosphoric acid derived NW arrays

For NWs from phosphoric acid templates in air, the transverse and longitudinal absorption peaks are at 429 nm 460 nm, respectively. Showing shifts of 3 nm and 7 nm compared to the oxalic-produced NWs, when inside the PAA template. Compared to oxalic-produced NWs in air, the reduction of absorption with increasing wavelength is significantly sharper, almost matching the oxalic-produced NWs inside the PAA template. The broad peaks at 520 nm and 700 nm closely match that of the oxalic NWs in the PAA template. This is consistent with the larger separation of the structures from phosphoric-produced NWs at 205 nm compared to the 100 nm of oxalic-produced NWs, and the larger relative permittivity of alumina compared to air, of 2.25[36] and 1, respectively.
5.3. RESULTS AND DISCUSSION

Figure 5.16: Absorption spectrum of metal NWs arrays, produced using phosphoric acid at 40 V without Left and with Right additional low pass signal processing. For phosphoric acid the transverse and longitudinal absorption peaks are at 429 nm 460 nm, respectively. Two broad NW–NW coupling peaks at 520 nm and 700 nm can be seen, separated by a dip in absorption.

This shows that the electronic properties of oxalic-produced NWs inside the PAA template can be replicated using the larger phosphoric structures, while in air. This is hugely significant, since at the intended 450 nm excitation wavelength, PAA is fluorescent[29], and would create a significant broadband-fluorescence background signal. It also suggests that the reported properties for silver NWs in the oxalic PAA template, may be achievable with the larger phosphoric-produced NWs structures. These properties include a reported negative refractive index[22] and evanescence wave transport[2].
5.3.2 Microscopy

5.3.2.1 Oxalic acid derived NW arrays

Using transmission fluorescence microscopy allows the refractive and scattering properties of silver NW arrays to be directly assessed, for further details see section 5.2.2. The micrographs in figure 5.17 show direction dependent scattering at 450 nm. Oxalic 1 is anisotropically scattered along one axis, Oxalic 2 has four regions at 90° to one another, where no light was scattered into. This demonstrates strong diffraction properties, of the 40 V oxalic acid-produced NW array, outside of the PAA template. The presence of diffraction demonstrates a poor SP coupling into the array, and that light scattering is the dominant effect. This could have been caused by the 17% weaker absorbance at 450 nm of free oxalic-produced NWs, compared to NWs inside PAA.

![Figure 5.17: False colour optical micrograph of a microscope focus as control, and the focus of two parts of a 40 V oxalic acid produced silver NW array, outside of the PAA. The device causes broadening of the original focal spot due to diffraction from the structures. The differing structural order of the NWs and structural defects caused different patterns of diffraction.](image)

The far-field diffraction pattern is illustrated in figure 5.18 right, which has the same approximate form as a hexagonal close packed crystal, shown left. This further supports the conclusion that diffraction is the dominant NW–light interaction.

![Figure 5.18: Schematic diffraction pattern of a hexagonal close packed crystal[38] (left), and far-field diffraction pattern observed for the oxalic acid 40 V device (right).](image)
5.3.2.2 Phosphoric acid derived NW arrays

The optical micrograph in figure 5.19 shows the phosphoric-produced NW device improving the focusing properties of the control significantly. This is in stark contrast to the oxalic produced device, where mostly scattering resulted. This is supported by the focus profiles excited at 450 nm, displayed in figure 5.20. The non-diffraction limited control input beam, focus was 8.2 \( \mu \)m FWHM and the phosphoric NW device gave a 2.3 \( \mu \)m FWHM focus, a reduction by a factor of 3.6.

![Figure 5.19: False colour optical micrograph of a microscope focus as control and the focus of phosphoric 100 V device. The focal spot appears to have been reduced using the device, see the focus profile in figure 5.20.](image)

![Figure 5.20: Focus profile of microscope focus control compared to the focus of phosphoric 100 V device. The FWHM has been reduced by a factor 2.7 using the device. It is important to note that the focus is not diffraction limited in the control and that this device is only able to improve a non-diffraction limited beam.](image)
Comparing the structure of the oxalic and phosphoric produced NWs, may give insight into this dramatic shift in properties. Compared to the oxalic produced devices, the NW period increased by 105% from 100 nm to 205 nm, and the NW diameter increased by 197% from 64 nm to 190 nm. Both changes are very significant, but the NW diameter is the most significant both in terms of value and in terms of changing the device’s properties. It is known that the resistivity of planar thin films of silver drastically increase for film thicknesses below 100 nm[39], and that it has decreased to a plateau for thicknesses around 200 nm[39]. Therefore NWs with 190 nm diameter are likely to have much reduced resistance and improved efficiency for SP transport. This is supported by the absorption spectrum in section 5.3.1.2, where a heavily etched oxalic NW device was required in order to measure the absorption, but no etching was required for the phosphoric device.

5.3.3 Fluorescence correlation spectroscopy of oxalic NW array

To determine if there was a near-field focus with the oxalic device followed by sharp divergence a FCS experiment was conducted.

The absorbance spectra in section 5.3.1.1 show high absorbance of both the laser at 450 nm, and the fluorescence emission of rhodamine 110 at 530 nm. The low signal intensities from the emission of a small numbers of molecules (5–50), may be lost during transmission through the NW device. The excitation power was also greatly reduced by the same process. This is demonstrated in figure 5.21, where the NW device has negligible fluorescence correlation. There is also little structure to the trend, which appears to be mostly noise, when compared to control on glass.

While trying to measure FCS with the NW device, the number of photon count events per second was very close to the background level, at 5 KHz and 4 KHz respectively. This is equivalent to a signal to noise ratio of 1:5, which is highly unfavourable to getting clear results. The experiment was conducted using 100 nM of rhodamine 110, where the control measured photon counts per second of 330 KHz.
5.3. RESULTS AND DISCUSSION

Figure 5.21: Correlation graph for a control (blue) and a device (red) with rhodamine at 100 nM. The device’s trend somewhat resembles that of the control until $10^{-4}$ s where it deviates, likely caused by the low signal to noise ratio (1:5). The large absorbance of the device is clearly hindering the measurement of the signal.
5.3.4 Time correlated single photon counting

The lensing properties of the phosphoric and oxalic NW devices were correlated to the SP efficiency in those devices, with superior lensing properties present in the more efficient phosphoric produced NW device. In figure 5.22 it is demonstrated that the fluorescence lifetime is significantly more suppressed in the phosphoric NW device, than both the oxalic NW device and the control.

Figure 5.22: Fluorescence decay of rhodamine 110, in the near-field of a device (red, green), compared to control (blue). The device templates were made with H₃PO₄ at 100 V (red) and oxalic acid at 40 V (green). Illumination was through a rhodamine 110 solution onto the top of a device, in an epi-configuration. Lifetime suppression was most extreme with the 40 V oxalic acid device.

In the experiment reflection dominated the signal despite significant emission filtering and laser power reduction. In order to resolve the fluorescence lifetime decay curves a reflection control without fluorescent dye, at the same laser power, was recorded and the photon flux at the detector was recorded. The decay including the fluorescent dye and unwanted reflection was then recorded and the decays were normalised to their peak intensities and then according to the intensity ratio of reflection only to reflection and fluorescence decay the reflection curve was subtracted from the experimental curve. This is valid since any contributions are additive in the TCSPC histogram. Some artefacts have resulted before the main peak due to slight differences in the decay curves prior to the main
peak, these should not affect the fluorescence dominated portion of the curve, however.

The fluorescence intensity $I$ decay curves had very large residuals when fit with a single exponential function. They were fit successfully to a double exponential curve model, with an experimental background noise correction term $y$-offset, the equation is described below,

$$I = a \cdot \exp\left(-\frac{t}{\tau_1}\right) + b \cdot \exp\left(-\frac{t}{\tau_2}\right) + y$-offset \tag{5.3.1}$$

where $a$ and $b$ are the exponential pre-factors and $\tau_1$ and $\tau_2$ are the fluorescence lifetimes of the first and second components, respectively. The resulting fit parameters are summarised in table 5.1 below.

Table 5.1: Rhodamine 110 excited on the surface of phosphoric and oxalic produced NWs outside of PAA in TCSPC experiment. Table of the double exponential fit parameters, which estimate the two component Fluorescence lifetime in both cases. The control rhodamine 110 decay occurred by single exponential decay in an average of 4.1 ns.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phosphoric acid</th>
<th>Oxalic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter estimate</td>
<td>Std. error</td>
<td>Parameter estimate</td>
</tr>
<tr>
<td>$y$-offset</td>
<td>88.88</td>
<td>3.23</td>
</tr>
<tr>
<td>$a$</td>
<td>8007</td>
<td>80.59</td>
</tr>
<tr>
<td>$b$</td>
<td>2079</td>
<td>86.35</td>
</tr>
<tr>
<td>$\tau_1$</td>
<td>0.566</td>
<td>0.0067</td>
</tr>
<tr>
<td>$\tau_2$</td>
<td>2.447</td>
<td>0.0772</td>
</tr>
</tbody>
</table>

The control rhodamine 110 decay occurred by single exponential decay in an average of 4.1 ns. The phosphoric NW device fluorescence decay is 80% occurring via a 0.57 ns pathway with only 20% in the still relatively short 2.45 ns decay pathway. With oxalic NWs enhanced rhodamine 110 decay, the populations between the two pathways shift, as do the timescales of each pathway. Rhodamine 110 on oxalic NWs decays with a 57% probability by the 1.53 ns pathway and with a 43% probability by the 3.68 ns pathway.

The two pathways involved are likely to be a closely associated solution state and a
surface adsorbed state, with decay lifetimes proportional to the SP field strength at each position[40]. At a constant height the phosphoric NWs have a surface area which is a factor of 9.5 larger than the oxalic NWs, the structure is not precise, but approximately the packing density of oxalic NWs is a factor of 4 higher. Taking both of these factors into consideration, the phosphoric NWs have an active surface area a factor of 2.4 larger per unit area of the substrate they are mounted upon. The relative population ratios of the surface and solution decay pathway are 3.85 and 1.34 for phosphoric and oxalic NWs, respectively. The factor between the decay path ratios of phosphoric and oxalic NWs is 2.87, which is in approximate agreement.

The artefacts from residual reflection in the fluorescence decay were not able to be accounted for in the fitting, which resulted in large $\chi^2$ values for the fits. This was mainly caused by the region close to the peak but the remainder of the decay curves were fit relatively well, as shown by the residuals in figure 5.23. The peak height was 10000 counts and the standard deviation of the residuals is 26 counts (0.26% of peak) for the oxalic NW device, and 57 counts (0.57% of peak) for the phosphoric NW device.

Figure 5.23: Fit residuals to a double exponential model to the fluorescence decay of rhodamine 110, in the near-field of phosphoric and oxalic NW devices on the (left and right), respectively. All residuals are <0.6% of the peak height, however the residuals are structured in the case of the oxalic NWs. The time zero point has been adjusted to the peak of the fluorescence decay.
5.4 Conclusions and outlook

Nanowire absorption is problematic to many applications such as using the NW device for FCS. The reflection from the NW arrays is also significant and the increased laser filtering needed to detect the fluorescence over the reflection, nullified any potential gains in fluorescence intensity. It is potentially misleading to compare using the same filtering set, since the control does not require the same level of filtering. Fluorescence enhancement was found to not increase signal levels compared to a realistic control, but only degrade them.

The relatively organised structures have an approximately constant density allowing comparisons of the fluorescence lifetime which would otherwise not be valid for solutions based nanoparticles. This is because the density of nanoparticles has a strong effect on the properties[8]. From TCSPC measurements it has been shown that the fluorescence lifetime quenching is approximately proportional to the nanoparticle surface area.

The nanowire absorption spectra revealed parallels in resonance positions between oxalic NWs inside PAA and free-standing phosphoric NWs from a template anodized at 100 V. The peak positions are closely matched, with deviations of less than 10 nm across the spectra. This has enabled previously impossible fluorescence and lensing studies to be conducted at the 450 nm excitation wavelength, without background fluorescence from the PAA template.

Going against the literature consensus efficient plasmonic devices need not have deep sub-wavelength dimensions, provided the structures are smaller than half of the incident light wavelength. The efficiency of the SPs generated greatly increased for NW structures 190 nm in diameter, compared to 64 nm because of their greatly increased surface area. This allowed far field focusing using NWs to be demonstrated at 450 nm using SPs and operating using negative refractive index, for the first time. The focal diameter was reduced by a factor of 3.6 on a non-diffraction limited input beam, an important first reporting of the proof of principle demonstrated in the far field at 450 nm.

For the first time a nanoscopic optical focusing structure has been produced where the precision of the structure is not critical. The relative lack of structural order of the large phosphoric structures had no observable effect on the optical focusing properties. The
opposite is true for the conventional oxalic NW structures outside of the PAA template, where spatial variation in the NW ordering drastically changed the observed optical focusing properties. This could have far-reaching commercial applications in nano-scale or ultra-compact optics. Enabled because of the relatively low tolerances required and the compatibility with thin aluminium film processing on dielectric substrates.
BIBLIOGRAPHY


CHAPTER SIX

CONCLUSIONS AND FUTURE OUTLOOK
CHAPTER 6. CONCLUSIONS AND FUTURE OUTLOOK

The fluorescence enhancement applications are not the applications of interest because of the fairly low efficiency of the process for all-but the most inefficient starting systems[1]. Ultrasmall lenses however deserve more attention in order to determine the optimum structure. In terms of the NW diameter, structural period of the NW array and the optimum height to ensure low transmission losses. Potentially it could form a focusing front optic on a microscopy device to realise a flat-pack microscope for field-work requiring it such as forensic investigator.

To apply this lens to practical applications it would need to be passivated since silver reacts with trace amounts of sulfur in the air and forms silver sulfide[2] a less conductive inorganic compound. Silver is also a known antimicrobial[3], which could limit it’s application in cell research without passivation. Titanium diode is a strong candidate for passivation since it can be generated in solution[4], and is biologically compatible[5] and transparent.

If the device works well there is scope to explore transitioning to mass production, since all of the techniques applied are compatible. Including the metal evaporation, which is currently used in solar cell manufacture. Electroless silver plating has been used in traditional mirror manufacture, and anodisation is used industrially and in aerospace applications to protect or enhance aluminium products.

Future prototypes would explore if the lensing and negative refractive index properties could be replicated with a reduced absorption, using titanium dioxide instead of silver metal. This would not rely on the SP coupling in order to generate the effect, but only the scattering and subsequent constructive interference. The size regime and period of the structures is between what is normally used in plasmonics and photonic crystals and it shares the periodic properties found in photonic crystals. This research would aim to further blur the lines between these fields and would discover if the scattering or SP coupling is the dominant effect.


