PLASMONIC NANOPORES: exploring new possibilities in DNA sensing and trapping

FRANCESCA NICOLI

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Francesca Nicoli

Department of bionanoscience

Supervisor
Dr. Magnus Jonsson

Examiner
Prof. Cees Dekker
ABSTRACT

In this work we explore the new DNA sensing and trapping possibilities offered by a plasmonic nanopore. This new device combines the sensing capability of solid state nanopores with the effects arising from the collective oscillation of electrons in gold nanoparticles (plasmons). The device consists in a nanopore drilled in a silicon nitride membrane and triangular gold nanoparticles in a bowtie configuration co-aligned with the pore. We exploit plasmonic heating to characterise the metallic nanostructures and we study its effect on pore stability. These observations allow us to optimise the sample characteristics and the experimental conditions for DNA translocation experiments. In this regard, we focus on how plasmonic excitation influences the depth of a current blockade, evaluating the contribution of the shift of the resonance spectrum due to the presence of a molecule in the surroundings of the particles. We also forwarded the hypothesis of a contribution of thermophoretic forces, due to high local heating induced by the plasmonic structures. Heating and thermophoretic forces could also influence the increase in translocation frequency, observed in our experiments upon plasmonic excitation. This increase is probably due to changes in the buffer viscosity which affects the magnitude of the pore’s capture radius of the DNA molecules. We also explore the possibility of controlling the motion of DNA inside the pore by means of optical trapping. This principle is based on utilising optical forces from the strong gradient of the evanescent electric field that is created, upon illumination, from the surface of the metallic nanoparticles to few nanometers away. From preliminary molecular dynamics simulations, we expect the optical force to be able to overcome the driving electric force at experimentally relevant excitation powers. Indeed, for plasmonic excitation around laser power 10 mW and above, we observed long (> 1s) and multilevel events, which indicate successful plasmonic trapping of DNA in nanopore. However we note that such long events were often present also after turning off the plasmonic excitation, likely due to permanent sticking of to the pore/bowtie. Although we need to solve some issues of sticking of molecules and to perform more systematic experiments before drawing any conclusion regarding trapping, the results are promising and indicate that plasmonic nanopores may enable light-controlled trapping of DNA in nanopores.
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1 Introduction

In order to study complex biological systems, the interactions between molecules have to be understood and molecular structure has to be analysed. For this purpose great efforts on developing single-molecule techniques have been made in the past years, as proven by the increase of the number of publications about single molecule techniques.\(^1\) The two primary used approaches are fluorescence (for example: fluorescence imaging with one-nanometer accuracy or fluorescence energy transfer) and force spectroscopy (for example: optical and magnetic tweezers and atomic force microscopy). With these techniques, great achievements on the spatial, temporal and force resolution have been made.\(^1,2\) One of the biggest advantages of these single-molecule methods is that they do not require a population-averaging of the ensemble, but they are capable to resolve rare or transient phenomena.\(^2\) Furthermore they enable the possibility of correlating multiple observables.\(^1\) On the other hand fluorescence measurements and force spectroscopy require labelling of the target molecules with a probe. The labelling procedure is often difficult, expensive and time consuming and the interaction between the probe and the target can introduce artefacts and uncertainty in the data.\(^2,3\) To cope with this issue label-free techniques are promising.\(^3\)

Solid state nanopore measurements fall in the category of single molecule and label-free detection methods.\(^4\) A solid state nanopore is a hole in the order of 2 to 50 nm drilled in a synthetic insulating membrane (ex. silicon oxide, silicon nitride, aluminium oxide).\(^5,6\) When the nanopore is in between two isolated chamber filled with an electrolyte, ions, i.e. ionic current, can flow through upon the application of an electric potential. Charged molecules, such as DNA, can be threaded through the pore and this translocation is read as reduction of ionic current. The big picture behind DNA nanopores experiments is the dream to develop a method to sequence DNA in a fast, reliable and low-cost manner.\(^4,7,8\) This will enable the realization of ambitious projects such as the “personal genome project” and “the cancer genome atlas”.\(^4,8\) The former aims to provide complete genome and medical records of several volunteers and to develop tools for interpreting this information for personalised medicine. The latter aims to study the variation of single nucleotides correlated with major cancers. However single-base resolution has not yet been achieved with nanopore experiments, partially due to the short time taken by the DNA to translocate. So the control over the DNA translocation speed would be an important step towards achieving single-base resolution in DNA sensing.

In this work we try to develop a method that combines the label-free DNA detection by solid state nanopores with the optical trapping of single molecules, theoretically achievable by means of plasmonic nano-tweezers. Plasmonic tweezers use the evanescent electric field created, upon external illumination, in the gap of nanoantenna. When two metallic nanoparticles are brought close (few nm) together, the plasmons oscillation is coupled leading to the formation of a strong electric field in the gap between the particles. Furthermore, we are interested in studying the plasmonic effects on solid state nanopores measurements, concerning heating and sensing. We focus on how DNA translocations are affected and which improvements arise from the combination of solid state nanopores and plasmonic nanoantennas. Other devices that combine nanopore with plasmonic particles were recently developed with either sensing or trapping purpose. Regarding sensing, array-based sensors were fabricated by drilling pores directly in gold film,\(^9\) by etching silicon nitride and the gold film deposited on top\(^10\) or by depositing gold plasmonic structures inside nanopores in a synthetic membrane.\(^11\) These devices combine the optical properties of plasmonic sensing with the benefits of micro and nanofluidic, such as control of the flow rate and the
exchange of liquid containing the analyte. Furthermore the array-like configuration enables high throughput measurements. For trapping purposes a variety of configurations bringing together nanofluidics and plasmonics have been studied. Gold nanowires separated by a small gap were used to trap 200 nm dielectric particles and E.coli bacteria.\textsuperscript{12} Nanoholes or a cavity in a thin metal film were also proved to trap objects of few tens of nanometers.\textsuperscript{13-15} All these devices differ from our plasmonic pores in the sense that they are based on molecules diffusing in liquid and entering the radius of the trap only driven by Brownian motion. In our system we try to use two competitive forces: the electrical force driving the DNA through the pore and the optical force trying to pull the molecule. Furthermore our plasmonic chips combine the well studied measuring capabilities of nanopores with the plasmonic sensing and thermal properties, opening new prospective for nanopore-based devices.
2 Theoretical background

The first part of this section is an overview on solid state nanopores, with particular focus on the electrical characterisation and the forces involved in DNA translocations. The second part is focused on plasmonics.

2.1 Solid state nanopores

Nanopores are natural occurring entities, they are present on the cell membrane to control the exchange of substances between different compartment of the cell and between the cell and its surrounding environment, for example they control the exchange of ions, nucleic acids and other biological molecules. Measurements of ionic current passing through the biological channels is a routine type of measurement in biophysics and in 1996 Kasianowicz demonstrated that it is possible to pull polyanionic DNA or RNA through an alpha-hemolysis nanopore by applying a voltage across a lipid membrane where the pore is embedded. In this pioneer experiment they monitored the ionic current of the pore and they noticed that after adding poly-nucleotides on the negative side of the membrane, they were drawn towards the positive electrode by the applied potential and when going through the pore the molecules caused short current blockades. They proposed that the lifetime blockade could give information of the polymer length as well as on its structure. This opened the possibility of having a single molecule detection technique potentially capable of giving information on the composition and structure of the polymer, and in case of DNA, to directly determine its specific sequence, based on the idea that each nucleotide has a characteristic electrical signature. Although transmembrane proteins were the first type of pores to be used in DNA detection experiment, they have some important limitations: difficulties in controlling structural changes such as pore diameter and thickness and limited stability. One promising alternative to overcome these limitations is represented by solid state nanopores. Solid state nanopores are small holes (from few to tens of nm) in a membrane consisting for example of silicon (Si), silicon nitride (SiN) or silicon oxide (SiO₂). The pore is drilled by means of etching, ion-beam sculpting, electron-beam lithography or, as in this work, by a focused electron beam. Such pores exhibit superior chemical, mechanical and thermal stability over biological pores and their structure can be easily controlled thanks to the advanced and well established microfabrication techniques. Furthermore solid-state nanopores could be integrated into devices and arrays, increasing their potential in future applications.

The research on synthetic nanopores has expanded rapidly in the past years, motivated by the prospect of leading to the so called third generation DNA sequencing. The past and current methods to sequence DNA include Sanger’s method, fluorescent in situ sequencing and pyrosequencing. These techniques are successful and reliable, but they have several downsides such as large amount of sample required, labelling procedures and complex readout techniques. Single molecule sensing with solid state nanopores can help overcome some of these limitations enabling fast and cheap screening of the genome.
Electrical characterization and DNA translocations through a solid-state nanopore

Monitoring the amount of ions flowing through the pore gives us information on its structure (diameter, membrane thickness) and on the stability of the measured current. A constant current trace indicates a stable pore and by changing the applied voltage we can establish the conductance of the pore. From the conductance measurement we can fully characterise the pore. The typical electrical characterization is carried out by placing the chip between two electrically isolated chambers filled with monovalent electrolyte.\textsuperscript{6,17,18} A schematic of a flow cell is shown in figure 2.1. The design of the flow cell can change according to the type of experiment one wants to carry out. As in this work, for example, the access to a microscope is needed in addition to electrical measurements (see experimental setup section).

![Figure 2.1: Schematic representation of the typical flow cell for DNA translocation experiment. The chip containing the pore is placed between the two chambers filled with the electrolyte (KCl), DNA molecules are added in the cis-chamber and drawn towards the positive electrode through the pore. Figure adapted from ref 17.](image)

As electrolyte, 1M potassium chloride (KCl) buffer is commonly used, since the effect of surface charges can be neglected at high salt concentrations and the electrical signal is higher.\textsuperscript{19} Other concentrations and types of salt can be employed, as long as this fact is taken into account when interpreting the data according to current theoretical models, which include also the different bulk conductivity of the liquid.\textsuperscript{20,21} The voltage bias is applied by placing two Ag/AgCl electrodes on the opposite sides of the chip. A localised oxidation-reduction reaction takes place between the electrolyte and the electrode, at the anodic electrode Cl\textsuperscript{-} is absorbed while at the cathodic electrode Cl\textsuperscript{-} is released in the solutions,\textsuperscript{22} the electrons flowing towards the positive electrode are measured as current. A linear voltage-current behaviour is expected for an open pore i.e. a pore without any molecules inside or in the proximity partially blocking the ions flow. Furthermore the measured conductance should match expected values for the pore geometry (diameter and thickness) and the bulk conductivity of the buffer. The theoretical model of the pore conductivity assumed in this work was presented by Kowalczyk et al.\textsuperscript{20} They considered the pore to have a cylindrical shape and the total resistance to be the sum of two factors, the channel and the access resistance. They obtained the following equation relating the conductance, $G$, with the geometrical parameters of the pore, where $d$ is the diameter and $l$ the membrane thickness, and the bulk conductivity of the buffer, $\sigma$:\textsuperscript{20}

$$G = \sigma \left[ \frac{4l}{\pi d^2} + \frac{l^{-1}}{d} \right] \quad (2.1)$$
The thickness considered in this model and in our work is not the real membrane thickness, but an effective thickness of 8.6 nm. A more realistic description is to consider the pore having an hourglass shape, but we find that the cylindrical model is accurate enough for our purposes.

The electrical characterization of the pore is fundamental to establish if it is suitable for DNA translocations experiments. First of all the dimension of the pore needs to be suitable for the type of nucleic acids we want to detect: very narrow pores, about 2 nm, allow translocations of single strand DNA only, while double strand DNA can translocate through larger pores. The principle behind translocation of DNA is straightforward and is based on the fact that the backbone of the nucleic acid is negatively charged, so when the polymer is added in the negative cis chamber of the flow cell, the DNA is electrophoretically driven towards the positive electrode. When the polymer goes through the pore it partially blocks the flow of ions inducing current blockades i.e. the current value of the open pore is temporarily reduced. The standard readout of a measurement is shown in figure 2.2. The downwards spikes are the blockades, each one caused by the passage of a single DNA molecule, called event from now on. By studying the shape of the blockades, it is possible to distinguish two types of events: single-level and multi-level. The former can be categorized as the single molecule going through the pore in linear way and the latter corresponds to a folded DNA translocation.

Figure 2.2: Example of the electrical readout of a nanopore experiment. A) Current trace showing DNA translocations. The downwards spikes are the current blockades corresponding to a DNA molecule going through the pore. B) A zoom on a transient current blockade of a DNA molecule translocating in a linear fashion.

The time of translocation, called dwell time, is typically in the order of milliseconds and depends on different factors, such as length of the polymer, applied voltage, buffer type and concentration. The influence of the bias voltage on the translocation time comes from the fact that the force dragging the DNA through the pore is due to the applied electric field. The forces acting on the polymer in the pore will be discussed more extensively below, but a straightforward deduction can be made from the fact that the electrophoretic force is directly proportional to the applied voltage, so the translocation velocity and, consequently, dwell time will increase and decrease respectively, when a larger bias is applied. As presented by Fologea et al., the dwell time is inversely proportional to the applied voltage, as shown in figure 2.3. They carried out the measurements in 1.6 M KCl buffer and 20% glycerol in the range between 20-100 mV, which corresponds to the typical values employed in DNA translocation experiments.
Figure 2.3: DNA translocation time through a 6nm pore as a function of applied voltage, in 1.6M KCl buffer containing 20% or glycerol. The solid curve is the fit of the experimental data (points) with the mathematical relation $V^{-1/t}$ between voltage, $V$, and translocation time, $t$. Figure adapted from Fologea et al.\textsuperscript{26}

As for the relation with polymer length, the study of the dwell time distribution enables to discriminate between different DNA populations, because the duration of an event is larger for longer molecules. The mathematical relation between the time, $\tau$, and the DNA length, $L_0$, was found to be exponential according to the following formula:

$$\tau \sim L_0^\alpha$$  \hspace{1cm} (2.2)

The fit of the model to the experimental data, shown in figure 2.4, imply a value of $\alpha=1.27\pm0.03$.\textsuperscript{18}

Figure 2.4: Dwell time as a function of DNA length. The squares represent the experimental points and the line the linear fit according to equation 2.2 and $\alpha=1.27\pm0.03$. Experiments performed in 1M KCl, 10 mM Tris and 1 mM Edta at pH 8.0. Figure adapted from Storm et al.\textsuperscript{18}

Another important factor that affects the dwell time is the buffer type and concentration. As reported by Kowalczyk et al.\textsuperscript{21}, different counter-ions have different effects on the charge reduction of a DNA molecule, causing the forces acting on the molecule and, consequently, the translocation time to be different for different ions concentrations and type. They performed measurements in KCl, sodium chloride (NaCl) and lithium chloride (LiCl) buffers and found that the binding of the positive ions to the negative DNA backbone has a different duration for different ions. In fact K$^+$ ions binds for a shorter time than Na$^+$ and Li$^+$, with lithium being the ion that is bound for the longest time. This fact explains why a different translocation time is observed, as it is summarized in figure 2.5. In our experiments we firstly used KCl at 1M
concentration and then we moved to LiCl at 2M concentration, with the purpose of decreasing the electrophoretic force as much as we could.

**Figure 2.5:** Experimental points and linear fit of the translocation time of double stranded λ-DNA molecules as a function of the buffer concentration for different ionic species. Figure adapted from ref 21.

**Forces involved in DNA translocation through nanopores**

As illustrated above, there are several factors that influence the translocation time of a DNA molecule through a nanopore. These phenomenological observations correspond to different forces arising from distinct physical principles. First of all the primary force acting on the DNA and driving the molecule from the negative to the positive electrode is the electrophoretic force acting on the negative backbone of the DNA chain. Another factor that has to be considered is the formation of an electric double layer (EDL) on the pore’s walls. The walls are negatively charged and they attract positive ions that are driven towards the negative reservoir of the flow cell. This makes the liquid move in the opposite direction than the translocation. There are also entropic forces involved in the process of DNA uncoiling and recoiling and drag forces acting on the blobs of the polymer outside the pore. From all forces involved in the translocation process, there are two major forces governing the translocation process. These are the electrical force and the drag force acting on the DNA inside the pore. A schematic of their role in the translocation process is shown in figure 2.6. The calculated values of 113 pN 83.5 pN respectively are matched with experimental results obtained employing nanopores with diameters similar to those used in this work and same type of DNA (λ-DNA), so for simplicity only the electrical and drag forces will be considered in calculating the forces involved in our experiments.

**Figure 2.6:** Schematic of the two main forces playing a role in DNA translocation dynamics. The electrical force is counteracted by the dragging force acting on the DNA inside the pore. Figure adapted from ref 18.
The value resulting from the balance between the drag and the electrical forces was measured directly by means of optical tweezers, as illustrated in reference 28. Here it is assumed that when motion of the DNA is stopped, the electrical force, $F_{\text{electrical}}$, is perfectly balanced by the restoring optical force, $F_o$. The strength of the resisting force can be measured according to $F_o = k_{\text{trap}} \Delta Z$, where $k_{\text{trap}}$ is the optical trap stiffness and $\Delta Z$ the position of the trapped bead which can be directly measured. Similarly the electric force can be estimated with a straightforward calculation from $F_e = q_{\text{eff}} \Delta V/a$ where $q_{\text{eff}}$ is the effective charge of DNA, $a$ the distance between two base pairs and $\Delta V$ is the voltage applied across the pore. The results are summarized in figure 2.7.

![Figure 2.7: Force measured as a function of applied voltage for a single dsDNA molecule in a nanopore at 0.1 M KCl. In this model only the electrical force is considered and it assumed to act not on the bare charge on DNA, but on a reduced charge. The best linear fit of the experimental points (triangles, squares and circles) assumes a charge reduction of 72%. The inset is a plot of the slope of the linear fit at different buffer concentrations. Figure adapted from ref 28.](image)

Here the electric field is acting on the effective charge of the molecule and not on the bare charge. So balance between the different forces playing a role in the translocation process can be seen as the electrical force acting on the effective change of the DNA or as the sum of the electrical force acting on the bare charge and the drag force of the electric double layer ions moving in the opposite direction to the DNA, as we can see in figure 2.8.
Figure 2.8: Schematic of the cylindrical model assumed in the force calculation considering the electrical force acting on the bare charge of the DNA and the dragging force generated by the liquid opposing the motion of the molecule. Figure adapted from ref 29.

From this model it is possible to describe the electrical force as:

$$ F_{\text{electrical}} = \frac{2\pi e (\Phi(a) - \Phi(R))}{\ln(R/a)} \Delta V \quad (2.3) $$

Equation 2.3 directly relates the forces with the applied potential, $\Delta V$, the pore and DNA diameter, $R$ and $a$ respectively, and their surface potentials, $\Phi(R)$ and $\Phi(a)$. The assumptions in the calculation of the surface potentials and the spatial distribution of ions in equation 2.3 are Poisson-Boltzmann and Stokes equation respectively. In figure 2.9 the experimental dependence of the electrical force from the applied voltage and the pore radius is shown. The illustration included in 2.9 is a representation of how the force on the counter-ions is affected by a small and a large pore.

![Figure 2.9: Effect of the pore radius on the translocation dynamics. The dashed curve is the theoretical result considering the bare DNA charge, while the fit with solid line takes into account the difference of surface potentials (pore and DNA). The experimental points are obtained under the following conditions: triangles: 20 mM KCl, stars 33 mM KCl, diamonds 50 mM KCl. The schematic drawing represent the drag (yellow arrows) and viscous (blue and green arrows) force acting on the DNA in a small (4nm) and large (39nm) pore. Figure adapted from Van Dorp et al. 29](image)
Summary

As illustrated above, nanopores are useful tools for label-free DNA sensing. With pores of diameter below or above of about 2 nm one can allow translocations of either only single stranded or single and double stranded DNA. It is also possible to sort polymers based on their size with the simple electrical measurement and to discriminate between different poly-DNAs, which are DNAs strands made of only one oligonucleotide type. It is also possible to translocate proteins and other kind of polymers. Despite these achievements, in order to study local structures on polymers other methods, such as optical or magnetic tweezers are still needed, in the combination with nanopores technique. In such experiments the DNA has to be attached to a dielectric or magnetic bead. Concerning free-DNA, single base electrical sensing is yet not achievable. One of the factor is that precise control of DNA at the single base level is needed in order to be able to acquire the specific signal for each base.

Different approaches on how to slow down and control the DNA nanopore translocation have been proposed. In this work we studied one possible method based on optical trapping by means of plasmonic forces. The goal is to exploit forces generated by the strong electromagnetic field gradients in proximity of metal nanoparticles to control the motion of the DNA through the pore. The novelty of the method lies in the fact that it does not require polymer-bead conjugation, like it is needed for magnetic tweezers or optical trapping. The intensity of local optical fields with plasmonic nanoantennas can be four orders of magnitude greater than achievable by classical optical tweezers, enabling the trapping of object in the order of few nanometers, as discussed more below.
2.2 Plasmonics and nano-optical tweezers

This section provides a brief overview of the fundamental concepts in the field of plasmonics and how the optical forces generated by plasmonic excitations can be applied for optical trapping purposes.

Dielectric function of metals and definition of plasmons

To describe the behaviour of electrons in metals, a first approximation model is the free electron Fermi gas.\textsuperscript{33–35} The key assumption in this model is that the conduction electrons can move freely through the volume of the metal. The interaction between the electrons and an external electromagnetic field determines the optical properties of the metal. This interaction is well described by Maxwell equations in the classical regime.\textsuperscript{33} The electrons can be seen as cloud of negative particles moving against a fixed background of positive ions (atomic nuclei), under the influence of an electromagnetic field. This model is called plasma model, because separation of charges in the metal can be put in analogy with the state of matter called plasma.

All the optical properties of the material can be related to its dielectric function, $\varepsilon(\omega)$, which describes the motion of electrons in response to an applied electromagnetic field and it is defined in terms of dielectric displacement $D$, electric field $E$ and polarization $P$, as $D = \varepsilon_0 E + P = \varepsilon_0 \varepsilon E$. For our purposes it is convenient to go from the space-time domain to the Fourier space where we obtain a dependence of the dielectric function from frequency.

The expression for the dielectric function of a free electron gas, obtained directly from the equations of motion, is found to be:\textsuperscript{33,34}

$$\varepsilon(\omega) = 1 - \frac{n_e^2}{\varepsilon_0 m \omega^2} = 1 - \frac{\omega_p^2}{\omega^2}$$ \hspace{1cm} (2.4)

where $n$ is the electrons concentration, $m$ and $e$ their mass and charge respectively. The plasma frequency is defined as $\omega_p = \frac{n_e^2}{\varepsilon_0 m}$ and it is the quantity that separates the frequency range at which the dielectric function is positive or negative. The mathematical sign of the dielectric function is important when we consider the relation between $\varepsilon(\omega)$ and the refractive index of metals, $n$, $\varepsilon = \sqrt{n}$. We see that $n$ is imaginary when $\varepsilon(\omega) < 0$. Furthermore the dielectric function has a zero mode at $\omega = \omega_p$, which correspond to the longitudinal oscillation mode of the plasma, which in term of electrons in the metal means a uniform displacement of an electron gas. This leads to the definition of plasmons as a quanta of this charge oscillation.\textsuperscript{33,34,36}

In the above description the damping of electrons’ motion is neglected, but in a real system, the electrons constantly collide with each other. In order to get a better description the damping needs to be taken into account by introducing an extra term in the equations of motion, characterised by the collision frequency $\gamma = 1/\tau$, where $\tau$ is the relaxation time of the electron gas. By doing so we obtain the following complex dielectric function:\textsuperscript{34,36}

$$\varepsilon(\omega) = 1 - \frac{\omega_p^2}{\omega^2 + i\gamma \omega}$$ \hspace{1cm} (2.5)

This equation can be divided into a real and a complex part $\varepsilon(\omega) = \varepsilon_1(\omega) + i \varepsilon_2(\omega)$ that are respectively:
\[ \varepsilon_1(\omega) = 1 - \frac{\omega_p^2 \tau^2}{1 + \omega^2 \tau^2} \quad (2.6) \]
\[ \varepsilon_2(\omega) = \frac{\omega_p^2 \tau}{\omega(1 + \omega^2 \tau^2)} \quad (2.7) \]

Note that for large frequencies close to \( \omega_p \) the damping becomes negligible and equation 2.2 can be approximated with equation 2.1.

For \( \omega \gg \omega_p \) the free electron model predict the dielectric function to have a value close to 1, but this does not correspond to how noble metals, such as gold in our case, behave in reality. The first term in equation 2.5 can be replaced by \( \varepsilon_{\infty} \), which takes into account the positive polarization induced by the background of positive ions. So equation 2.5 becomes:

\[ \varepsilon(\omega) = \varepsilon_{\infty} - \frac{\omega_p^2}{\omega^2 + i\gamma \omega} \quad (2.8) \]

The dielectric constant \( \varepsilon_{\infty} \) typically has values between 1 and 10. This model is called Drude-Sommerfeld model and it accounts for the fact that conduction electrons do not oscillate in phase with the external field \( \varepsilon(\omega) \) has a significant imaginary part.\(^{35} \)

**Localised surface plasmons (LSPs)**

There are three main types of plasmons: volume plasmons, surface plasmons and localised surface plasmons. The first type is the one defined in the previous section. Surface plasmons are confined electromagnetic excitations of conduction electrons propagating at the interface between a dielectric and a metal and localised surface plasmons (LSPs) are non-propagating excitations of the conduction electrons of a metallic nanostructure coupled to the electromagnetic field.\(^{34} \) We specifically focus on the last type since we are working with gold nanoparticles.

In a metallic nanoparticle the electrons cannot propagate in the same way as at a metal dielectric interface due to its finite dimension. The electrons inside the particle are coupled to the electromagnetic field, as depicted in figure 2.10. The boundaries of the particle exert a restoring force on the oscillating electrons, giving rise to a resonance phenomenon, i.e. the electrons tend to oscillate with a greater amplitude at a certain given frequency (resonance frequency).\(^{34} \)
The resonance frequency depends on the shape and dimensions of the particle as will be discussed in section 2.3 specifically for our plasmonic structures. The electromagnetic field is amplified both inside and near the particle’s surface. These non-propagating excitations of electrons are called localised surface plasmons and they can be excited by direct light illumination at the resonance frequency. The oscillation of the electrons inside the metal generates electromagnetic fields inside and in proximity of the particle. The latter are strong evanescent fields confined to a small region around the particle’s surface.\textsuperscript{34,36}

If the dimensions of the particle are considerably smaller that the wavelength of the light in the surrounding medium, we can use the quasi-static approximation to describe the interaction between the metallic particle and the electromagnetic field. The approximation assumes that the phase of the incident field is constant over the particle volume, i.e. the problem can be simplified as a particle in an electrostatic field. The interaction between the particle and light can be described in terms of the polarizability, $\alpha$, defined as $p = \varepsilon_0 \varepsilon_m \alpha E_0$, where $p$ is the dipole momentum induced by the static electric field $E_0$, $\varepsilon_0$ and $\varepsilon_m$ are the dielectric constant of the vacuum and the surrounding medium in the particle, respectively. For a small spherical particle, $\alpha$ is given by:\textsuperscript{34–36}

\begin{equation}
\alpha = 4\pi a^3 \frac{\varepsilon - \varepsilon_m}{\varepsilon + 2\varepsilon_m} \tag{2.9}
\end{equation}

where $a$ is the radius of the particle and $\varepsilon(\omega)$ is dielectric function of the particle’s material. The polarizability has a maximum for $|\varepsilon + 2\varepsilon_m|$ being a minimum, which, for metals such as gold that have an imaginary part of $\varepsilon$ much smaller than the real one, implies:

\begin{equation}
\text{Re}[\varepsilon(\omega)] = -2\varepsilon_m \tag{2.10}
\end{equation}

In the calculation has been assumed that the particle is a sphere. If we are dealing with particles with a different shape, like our triangular geometry, the polarization of the incident light needs to be considered. Effects such as enhanced absorption and scattering and shift in the resonance wavelength arise.\textsuperscript{34}

Equation 2.10 is also called the Fröhlich condition and can be used to explain the basic behind the concept of LSP resonance sensing. The particle polarizability depends on the dielectric constant, consequently on the refractive index, of the surrounding medium. Small changes in $\varepsilon_m$ caused by the presence of a
molecule close to or on the particle surface will cause a shift in the polarizability maximum and in the resonance frequency. By monitoring this shift one can sense the presence of the molecule.\textsuperscript{34,36}

**Heating effects**

We want to take a closer look at the absorption cross section, because its physical effect is to cause heating of the metal.\textsuperscript{37} The power, \( Q \), absorbed by a metallic nanoparticle is proportional to the absorption cross section, \( \sigma_{abs} \), and the irradiance \( I \) of the incoming light according to:\textsuperscript{37}

\[
Q = \sigma_{abs} I \quad (2.11)
\]

and the absorption cross section can be expressed as:\textsuperscript{37}

\[
\sigma_{abs} = k \, \text{Im} \left( \alpha \right) - \frac{k^4}{6 \pi |\alpha|^2} \quad (2.12)
\]

where \( k \) is the wave vector of the exciting field. The heating effect has many different applications, such as photothermal imaging, photothermal drug delivery and photothermal therapy for local hyperthermia.\textsuperscript{37} For example, we use it as the mean to localise the pore on our membrane and to co-align the laser beam with the pore. In our experiments the plasmonic heating causes a temperature increase of the buffer of thens of degrees Celsius, depending on power and plasmonic structures.\textsuperscript{38} In general, probing the actual temperature around a plasmonic structure is not an easy task and requires very specific techniques. The methods used are based on fluorescence detection, for example, on monitoring the change of fluorescence anisotropy\textsuperscript{39} or fluorescence lifetime.\textsuperscript{40} Temperatures of plasmonic heating typically range from 20°C to 70°C depending on the incident laser power\textsuperscript{39} and different applications require different temperatures, for example for hyperthermia 40-42°C.\textsuperscript{39,40}

Plasmonic heating can also be used for interesting applications in opto-fluidics\textsuperscript{41} and in nanopore translocation experiments to achieve thermophoretic manipulation of the molecule.\textsuperscript{42} Regarding this last aspect, molecules tent to move along temperature gradients from the hot area to the cold one. For a theoretical modelling and mathematical derivation see reference\textsuperscript{43}. For DNA translocation through nanopores it has been theoretically calculated\textsuperscript{44} that the thermophoretic force can drive DNA molecules from a hot (cis chamber) to a cold (trans chamber) reservoir and untwist the polymer while translocating. The model is based on highly localised source of heat around the pore. In this respect plasmonic particles in proximity of a nanopore could fulfil the practical realization of having strong temperature gradients highly localised in space.\textsuperscript{42}

**Two-particle coupling: optical nanoantennas**

For our purposes it is also important to discuss the electric field distribution around the plasmonic nanostructures. In the case of the quasi-static approximation the metallic sphere behaves like a point dipole and the near field is predominantly electric.\textsuperscript{35} Interesting effects arise when two, or more, metallic particles are brought in close proximity and highly localised and strongly enhanced near fields are generated.

If two plasmonic nanoparticles are separated only by a small gap their resonance modes overlap both optically and spatially. This generates new resonances.\textsuperscript{34,35} An example of how the resonance wavelength changes with coupling of two gold spherical nanoparticles is shown in figure 2.11.
Figure 2.11: Resonance spectra of the gold nanoparticle with different gaps in between at different polarizations. The coupling between electrons changes with the gap size causing a shift in the resonance peak. Also the polarization of the incoming electric filed plays a role in shifting the spectrum. The oscillation of the electrons changes if they move along the major or minor axis of the particles ensemble. Figure adapted from Rechberger, W. et al.

To describe the behaviour of a system made of two nanoparticles we can borrow the mechanical model based on harmonic oscillators. We know that the external electric field generates oscillating electric charges on the particle's surface, so each resonator can be represented as a spring with the corresponding mass attached to it. When two particles are aligned and brought close to each other an additional spring has to be considered in order to account for the interaction between the charges on both particles. A schematic picture of the model and the resulting modes is illustrated in figure 2.12. The oscillation can be either in-phase or anti-phase. This fact generates two separate states, or new resonances, with an energy splitting. In the bonding, or in-phase, state the two individual charge oscillations are dipole-like. The resonance is red-shifted compared to the isolated particle and the state can be excited by plane-wave illumination. The anti-bonding, or anti-phase, state cannot be excited in far field spectroscopy and it does not emit in the far field because the dipoles oscillating out of phase cancel each other, such that there is no net effects. The optical properties of the particles ensemble can be tuned by controlling the coupling between the particles which depends on their spatial gap. It is worth mentioning that the polarization of the exciting electric field has a strong influence on the plasmonic spectrum of the absorbed and scattered light. The position of the spectrum shift depends on the direction of electrons excitation in the particle and on the coupling between them, see figure 2.11.
Figure 2.12: Spring model used to explain the coupling of electrons between two plasmonic particles. The two modes arising with the respective excitation spectrum are calculated for a 16 nm and a 6 nm gap between the structures. Figure taken from ref 35.

The type of structures described above can confine electromagnetic radiation to a very small region of space, called hot spot, and release it in the far field acting in a very similar way to a RF antenna. The plasmonic structures have a different emission pattern and a different spectral range compared with classical antennas, but they can be defined as optical nanoantennas to the extension of their ability to enhance and localise propagating fields of plane waves. The antennas we use in this project have a bow-tie configuration, consisting of two gold triangles facing each other tip to tip separated by a small gap (see figure 2.14 in the next section). They combine sharp metallic tips and coupled plasmonic particles properties. The field is concentrated in the gap and its intensity enhancement strictly depends on the gap size. A common method to predict the near field distribution and intensity around the particles is to use finite-difference time-domain (FDTD) simulations. Schuck et al. determined the enhancement experimentally for different bowtie gaps and compared the measured values with the ones predicted by the simulations. They obtained the experimental points by comparing the photoluminescence of the particles with the one of a gold film. The experimental results, summarised in figure 2.13, are in good agreement with the simulations and show an enhancement $> 10^6$ of the square of the electric field intensity.  

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Forces near an optical nanoantenna\textsuperscript{46,49}

For our purposes it is important to determine the optical forces generated by the interaction between and object and the near fields of the antenna. First we discuss the forces in electromagnetic fields felt by a particle which dimensions are smaller than the wavelength of light (Rayleigh particle). In this case we need to consider the polarization of the particle to calculate the force, which consists in a dipole force and a scattering force. The contribution of scattering can be disregarded, because there is no radiation pressure coming from the near field. The dipole force causes the polarized particle to move along the increasing gradient field. The force $\mathbf{F}$ felt by a Rayleigh particle is:

$$\mathbf{F} = \frac{\alpha}{2} \nabla E^2 \tag{2.13}$$

where $E$ is the module of the plasmonic electric field and $\alpha$ the polarizability of the particle. In case of plasmonic nanoantennas the electric field that needs to be considered is the one generated by the oscillating charges in the metal. In order to minimise the dipole potential energy the particle moves towards the region of high field intensity. In the Rayleigh approximation used to derive equation 2.13, it was assumed that the field is homogeneous across the particle and that the trapped particle does not alter the electric field. To get a better representation of the force in realistic systems Maxwell’s stress tensor, $\mathbf{T}$, has to be calculated and averaged over time. The derivation of the force using Maxwell’s stress tensor is beyond the scope of this work, so we refer the interested reader to the work of Novotny et al.\textsuperscript{49} for the complete calculation.
Optical trapping – advantages of plasmonic tweezers

Optical tweezers are a tool wildly used to manipulate and trap micro-scaled objects, with important application in, for example, the field of bioscience. The underlying principle is the one described in the previous section according to which a polarized particle tends to move toward the higher intensity of an electrical field gradient. In the case of classical optical tweezers, the electric field is the one of a tightly focused laser. Although it is still a resourceful tool, classical trapping presents several limitations. As the size of the trapped specimen is reduced, the magnitude of the trapping forces decreases and the Brownian motion of the particle increases. Hence a very small particle can have enough thermal energy to escape the shallower trap. Two ways to compensate for these effects are to further focus the laser and increase the field intensity. The former is limited by the diffraction limit of the optics, while the latter solution is not applicable in the case of biological samples or samples sensitive to light, because at high laser powers they may get damaged and/or destroyed.\textsuperscript{12,31,32}

Using evanescent plasmonic fields represent an alternative to overcome the limitation of classical optical tweezers. Optical nanoantennas, as described in the previous section, are capable of focusing the light beyond the diffraction limit by concentrating the electromagnetic field in the hotspot; allowing a reduction of the trapping volume and consequently an increase of an order of magnitude of the confinement. Also the laser power needed for the trapping is significantly reduced since both the intensity and the intensity gradient of the plasmonic field are enhanced. Trapping events of small objects in plasmonic nanotweezers are typically monitored by optical methods. These techniques measure the change in transmitted light\textsuperscript{12} or follow the path of the previously labelled object by detecting its fluorescence.

Several trapping configurations have been explored so far, such as optical cavity\textsuperscript{14}, nanostructured surfaces\textsuperscript{50}, nanoapertures\textsuperscript{15} and optical antennas\textsuperscript{12}. The trapped species are beads, metallic nanoparticles, bacteria and proteins.
2.3 Plasmonics meets nanopores

In this project we bring together the DNA sensing possibilities of solid state nanopores with the versatile properties of plasmonics nanoantennas.

Our plasmonic chip consists of a silicon chip with a free-standing silicon nitride membrane, 20 nm thick, on top of which an array of gold nanoparticles is fabricated by e-beam lithography. A nanopore is then drilled in the centre of a bowtie using a focused electron beam of a transmission electron microscope (TEM). An optical microscope image of the chip with the membrane, a scanning electron microscopy (SEM) image of the membrane and bowties and a TEM image of the bowtie where the pore is drilled are shown in figure 2.14.

![Image of chip with membrane and bowties]

**Figure 2.14:** A) Optical microscope image of the portion of the chip with the SiN membrane (in dark green) and the gold arrows used to localise the membrane when imaged with our CCD camera. B) SEM image of the SiN membrane (in dark grey) with the array of gold bowties (in light grey). C) TEM image of the bowtie where the pore was drilled.

The particles are shaped like bowties with a designed tip-to-side length of the triangle ranging from 50 to 120 nm. The standard measure is 60 nm because is the one for which the peak of the resonance curve of the plasmonic particles matches our laser wavelength of 785 nm. Samples with different bowtie lengths were fabricated with the purpose of better exploring the role of different plasmonic properties in our DNA translocation measurements. In fact, as explained in section 2.2, by changing the structure of the particles we can tune the resonance of our nanoantenna and monitor how this change affects the outcome of our experiment. When the electrons inside the bowties are excited by light they generate a strong evanescent electric field in the gap between the two triangles. Recalling the theory about nanoantennas, we can say that our system of two gold particles falls into that category. In figure 2.15 the simulated distribution of intensity enhancement of the evanescent electric field compared to the incident field is shown. The geometrical parameters of the bowties used in the simulation are: 60 nm tip-to-side length, thickness of 30
nm and 10 nm gap with a drilled pore of 10 nm in diameter. No adhesion layer between the gold and the SiN is considered and the membrane thickness is set to 20 nm. The incident electric field is longitudinally polarised, according to how the longitudinal polarization is defined in figure 2.16. As we can see the pattern of the radiated electric field follows the geometrical shape of the gold structures and the higher intensity is concentrated in the gap between the triangles (hotspot).

![Simulated distribution of the evanescent electric field around the gold bowties (the colour bar shows the intensity enhancement in logarithmic scale). The parameters assumed in the simulation are specified in the text.](image)

**Figure 2.15:** Simulated distribution of the evanescent electric field around the gold bowties (the colour bar shows the intensity enhancement in logarithmic scale). The parameters assumed in the simulation are specified in the text.

![Sketch of the gold bowties with the convention of longitudinal and transverse polarized light. When we refer to longitudinal polarization we consider the long axis of the bowtie. The transverse polarization refers to the short axis of the bowties.](image)

**Figure 2.16:** Sketch of the gold bowties with the convention of longitudinal and transverse polarized light. When we refer to longitudinal polarization we consider the long axis of the bowtie. The transverse polarization refers to the short axis of the bowties.

The idea behind combining nanopores with plasmonics is that the different properties of metallic nanoparticles can be exploited to improve the current applications of nanopore sensing and add more to it. The plasmonic effects investigated and utilised in this work are (i) the heat generated by the absorption of radiation by the gold particles, (ii) the shift in the plasmonic resonance in the presence of biological molecules and, last but not least, (iii) the force generated by the gradient of the intense localised evanescent field in the bowties hotspot.
The plasmonic heating can be exploited for profiling the focal volume of low power laser beams\textsuperscript{38} and, for our experimental purposes, for locating the pore on the membrane. The principle of this type of measurement is based on two concepts combined: the heating of the gold particles when struck by laser light and the dependence of the pore’s ionic current on the buffer temperature. From formula 2.1 we know that the conductance, consequently the ionic current, of the pore is proportional to the bulk conductivity of the buffer, which can be approximated as linearly dependent on the temperature according to:

\[ d_{\text{bulk}}(T) = a + bT \quad (2.15) \]

This means that the increase in conductivity/ionic current is also directly proportional to the temperature increase, see the section on heating experiments for a more detailed discussion between increase in ionic current and temperature. We also know from equation 2.11 that the heating, i.e. the increase in temperature, is directly proportional to the power of the incoming light. This means that the plasmonic nanopore can be used as a device to localise the maximum power of the incoming light that corresponds to the laser focus. This technique is used in our experiments to achieve a precise alignment between the laser beam focus and the bowtie-nanopore, to maximise the evanescent electric field. Furthermore, the temperature increase gives us an idea of the fabrication quality of our bowties, which directly affects how well the plasmons can be excited by our laser. The absorption cross-section is the parameter that reflects how much light that is absorbed generates heating, depending on the dimensions of the bowties and the polarization of the incident electric field. In figure 2.17 the simulated absorption cross-section of different bowtie lengths for the longitudinal and transverse polarization is plotted. The dashed vertical line represents our laser wavelength. The cross-section that is maximised at that value is the one for 60 nm bowties illuminated by longitudinally polarized light. We therefore expect to get the maximum heating for the 60 nm structures.

\[ x \times 10^{-14} \]

\[ 4 \]

\[ 3.5 \]

\[ 3 \]

\[ 2.5 \]

\[ 2 \]

\[ 1.5 \]

\[ 1 \]

\[ 0.5 \]

\[ 0 \]

\[ 650 \]

\[ 700 \]

\[ 750 \]

\[ 800 \]

\[ 850 \]

\[ 900 \]

\[ 950 \]

\[ \text{absorption cross section (m}\textsuperscript{2}) \]

\[ \text{wavelength (nm)} \]

\[ 50 \text{ nm} \]

\[ 60 \text{ nm} \]

\[ 70 \text{ nm} \]

\[ 80 \text{ nm} \]

\[ 90 \text{ nm} \]

\[ \text{Figure 2.17: Simulated absorption cross sections of bowties of different tip-to-side length. The different colours correspond to different length of the bowties long axis. The full and the dashed line spectra correspond to the cross sections for longitudinally and transversely polarized incident light, respectively. The vertical dashed black line is the laser wavelength.} \]
As mentioned in the description of solid state nanopores, one of the limitations for studying local structures on the DNA and for reaching single base resolution with this technique is the very short time taken by the molecule to translocate. One of the most interesting aspects of plasmonic nanopores is therefore to explore if the forces generated by the field gradient in the bowtie hotspot can be used for optical trapping of biomolecules as small as double stranded DNA. The advantage of combining nano-optical trapping with nanopores is that the DNA molecule we want to trap is electrically driven towards the pore and the plasmonic antenna by the electrophoretic force and not by simple diffusion. This guarantees that the molecule comes close enough to the hotspot in order for it to feel the plasmonic force. Then it is constrained to change conformation and translocate though the pore passing in close proximity of the particles and the plasmonic electric field. Preliminary molecular dynamic simulations for an incident electromagnetic field in the order of 10mW suggest that the plasmonic field should be strong enough to overcome the electrophoretic force and capture the DNA molecule for a longer time that what is needed by the molecule to translocate.
3 Experimental setup and procedure

In this section the sample preparation will be described together with the setup, including the flow cell design and the microscope light path. This is followed by a brief overview on standard measurement procedures, such as electrical characterization and pore localization. The variations within experiments will be included in the analysis and discussion section regarding the specific measurement.

3.1 Sample preparation

Schematic illustration of a sample is shown in figure 3.1. It consists of a silicon chip with a free standing silicon nitride membrane 20 nm thick. There is a 500 nm thick SiO$_2$ layer below the SiN membrane to reduce the dielectric noise in the nanopore. This type of noise is related to the capacitance of the nanopore chip that can be reduced by addition of the extra insulating layer.$^5$ We refer to the chip as having two sides: one, called the membrane side, is flat side of the chip, where the SiN is deposited; the second, called the pyramid side, is the one where the silicon got etched until the SiN layer.

The sample needs to be thoroughly cleaned before it can be used for a measurement. The first step is to rinse the chip with chemicals, specifically with acetone, isopropanol and ethanol, and dry it with a nitrogen gun. Then it is treated with oxygen plasma for two purposes: removal of contaminants and wettability of the pore. The chemically activated oxygen, O$_2^\cdot$, breaks down the organic bonds in molecules on the surface of the chip and forms volatile compounds that can be evacuated from the chamber according to $C_xH_yO_z + (O_2^\cdot, O) \rightarrow CO_2 + H_2O$, resulting in an ultra-clean surface.$^{51}$ Furthermore the plasma makes the pore hydrophilic enhancing the wettability of the pore.$^{19}$

After cleaning the chip is glued onto the flow cell with poly-dimethylsiloxane (PDMS) glue which is impermeable to liquid. This characteristic is important because it allows the two chambers of the cell to stay isolated from each other. Next, the two compartments of the flow cell are filled with buffer. Two types of buffer are used in this work: a solution of 1M potassium KCl, 10 mM Tris and 1mM of ethylenediamine-tetraacetic acid (Edta) at pH 8 and a 2M lithium chloride (LiCl), 10mM Tirs and 1mM Edta at pH 7.8. The first buffer is commonly employed in DNA translocation experiments, while the second one is used by us to decrease the electrophoretic force and therefore facilitate the trapping process.
Figure 3.1: Schematic representation of the A) Si chip B) SiN membrane with the bowtie array, C) pore and the gold bowties. Note that the edges of the gold particles are rounded in the reality. The x-y plane is the plane of the membrane and the direction are defined according to the bowtie orientation.

3.2 Flow cell

In our experiments we use a custom made flow cell, which is displayed in figure 3.2. The two main elements forming the cell are the cylinder, where the chip is glued onto, and the bottom compartment covered with a glass slide. The sample is glued with the pyramid side facing the inside of the cylinder, allowing the membrane side to go as close as possible to the glass of the lower compartment when the cylinder is pushed in. Both sides are filled with buffer so that the chip and the SiN membrane are properly wetted. At this point, as described in the theory part, the only channel for the salt ions to go from one chamber to the other is the pore. The flow cell is then mounted on an inverted custom-build microscope, so that the microscope objective can come close to the glass slide and, consequently, the laser can be focused on the membrane and on the plasmonic nanopore.

Two different approaches are used for our experiments on DNA translocation: the molecules are either added to the top or to the bottom compartment. With the former method the chip is simply glued on the
cylinder by putting the PDMS only around the hole on the cylinder. When DNA is instead added in the bottom compartment an extra layer of glue on two sides of the chip is needed to optimize the flux (see figure 3.2 A and B). We came to this conclusion by observing that the DNA events rate was extremely low when the sample was added on the bottom compartment, because the liquid tends to flow on the sides of the chip instead of underneath, preventing the DNA from ending up within the capture radius of the pore.

![Figure 3.2: Schematic drawing of the flow cell. A) View of cylinder where the chip is glued on. B) Bottom view of the cell, the chip is visible through the glass slide. C) Assembled flow cell, the cylinder is pushed all the way down until the chip is few um from the glass. The small arrows indicate where the electrodes are placed.](image)

3.3 Optical setup: inverted microscope and laser light path

The optical system used in our experiment is shown in figure 3.3. The laser diode emits in the near-IR region, specifically at 785 nm. This is a wavelength that is suitable for the resonance frequency of gold plasmonic antennas. Right in front of the laser there is an optical insulator which prevents the light from going back into the laser. In combination with a polarizing beam splitter it is used to control the power of the beam that effectively reaches the sample. A second half-wave plate is present after the beam splitter, to manually switch between transverse and longitudinal polarization during the experiments. After this step, the beam is expanded by a system of two lenses: the first, with a short focal length, focuses the beam to a precise spot, corresponding to the focal plane of the second lens, with a longer focal length, which collects the light and collimate the beam. This expansion is needed so that the spot can be tightly focused by the objective. The expanded beam is reflected by a mirror, oriented at 45°, and then goes to a beam splitter that allows the light to go to go through the system of mirrors, which guide the beam to the objective. The beam reflected by the sample goes back into the camera. The microscope consists of a water immersion objective and a piezoelectric stage. The objective has a numerical aperture, NA, of 1.2 and focuses the light to a spot of about 0.4 μm in diameter $D$ according to $NA \approx 2\lambda_0/\pi D$, where $\lambda_0$ is the laser wavelength in vacuum. The flow cell is mounted on the piezoelectric stage which allows us to precisely control the movements of the sample with respect to the laser focus. The optical system is completed by two extra light sources. The first is called the backside illumination because it illuminates the chip from the
backside. It consists of a regular white LED lamp and is used to localize the membrane. The second is also a white LED light which illuminates the top of the sample and is used to visualise the membrane during experiments. The image is formed on a CCD camera connected to the computer with a custom made Labview programme.

![Schematic of the optical path of the laser beam (in red) to the microscope objective and of the reflected beam form the sample to the CCD camera. The optical elements in the schematic are: (1) optical insulator, (2) mirrors, (3) polarizing λ/2 wave plate, (4) polarizing beam splitter, (5) λ/2 wave plate, (6) power meter, (7) flipping mirror, (8) lens with 19 mm focal length, (9) semi-transparent flipping mirror, (10) lens with 100 mm focal length, (11) beam splitter, (12) tube lens.](image)

### 3.4 Electrical setup and noise minimization

The equipment for electrical measurements consists of patch clamp amplifier including a pre-amplifier that the Ag/AgCl electrodes are connected to. The electrical apparatus can be brought in contact with the ionic solution in the flow cell using two different connectors: agarose salt bridges or Ag/AgCl electrodes. Our original choice was to use salt bridges, since they are typically less affected by laser light than Ag/AgCl electrodes. Instead after our measurements of the noise level we came to the conclusion that the latter generate less noise than the salt bridges for our measurements. Hence the final choice was to use Ag/AgCl
electrodes. To minimize electrical noise, the microscope stage, including the flow cell and the pre-amplifier, is surrounded with a copper box, acting like a Faraday cage, which main purpose is to isolate the inside from external electromagnetic fields.

After being amplified the current is measured by an amplifier, specific for patch clamp measurements. We use the voltage clamp method, commonly used to detect bioelectric signals. By keeping the voltage constant, or changing it in steps of discrete values, we are able to monitor the current i.e. the conductance of the nanopore, similar to what is done to determine ion-channel activity in biological membranes.

The electric output needs to be filtered to reduce noise and unwanted signals, for example electrical fields coming from the power system of the setup. We use a low-pass Gaussian filter to remove the part of the signal above a chosen frequency, typically 2 kHz. This enables us to detect DNA translocations, which may otherwise be obscured at higher or no filter frequency.

We observed an increase in noise caused by the laser beam hitting the sample. This increase in noise was not due to the effective power (number of photons) reaching the sample, but was found related to the laser output power. As shown in figure 3.4, at very low laser outputs, the standard deviation of the current increases much more than for high outputs. This is probably due to laser beam instability at low powers, so in our experiments the laser output is kept constant at 80 mW and the power is controlled by the polarizing beam splitting method.

![Figure 3.4: Increase in the standard deviation of the current (I) as a function of laser power at different laser outputs. Signal filtered at 10 kHz.](image)

*Figure 3.4: Increase in the standard deviation of the current (I) as a function of laser power at different laser outputs. Signal filtered at 10 kHz.*
3.5 Electrical measurements and pore localization

After the sample is prepared and the flow cell is placed on the microscope, we perform an electrical characterization of the pore to determine if it is suitable for our experiment. First of all we want to determine the resistance of the pore and the linearity of the IV curve. The resistance values for a good pore should match the expected value for the drilled pore diameter (5 to 20 nm) at the given buffer. As illustrated in the theory section the resistance vary with the type of buffer used. The linearity of the IV curve is also checked. Very high resistance values combined with non-linear IV curve indicate that something is blocking the pore. Resistance in the order of 100 MΩ means that an air bubble is probably on the pore. Furthermore, if the pore will be used for DNA translocation experiments, the measured ionic current trace needs to be stable and the noise has be low enough to detect events. In order for the current blockades caused by the DNA translocations to be detectable, the acceptable maximum value of the current standard deviation should be about 40pA.

Once that outcome of the electrical characterisation indicates a pore with the right characteristics, the following step is to localise the membrane on the chip, and then the pore. First of all the laser is focused on the chip, then the membrane is found easily using the backside illumination and moving the stage manually with the micrometric screws. Arrows pointing in the direction of the membrane are fabricated to facilitate the localisation process. Once the membrane is found, it is moved over the laser beam in the x-y plane (defined in figure 3.1-B) while the pore’s ionic current is monitored. This procedure enables us to find the pore, because, as illustrated in the theory section, when the laser hits the bowties it excites the plasmons. This leads to a local enhancement of the temperature, which causes a local change in the buffer conductivity. If the bowtie hit by the light is the one with the pore in the gap, the heating of the buffer results in an increase of the measured current compared to the value obtained in the absence of plasmonic excitation (base line value). The precise location of the nanopore is determined by multiple x-y scans of progressively smaller areas. The exact laser focus is found exploiting the same principle, in the z direction. An example of such scans is shown in figure 3.5. It is worth noting that the current distribution does not correspond to the shape of the pore, but it is a representation of the laser beam. In fact in this type of measurement the pore is the effective scanning probe of the beam.38

We can get further information on the structural quality of the bowties from the scanning measurements by calculating the relative change in the pore conductance. The value is estimated by dividing the maximum current by the current baseline and it gives us an idea of how efficiently the plasmons are excited by the laser light. The greater the change in conductance the more efficient the excitation is. The efficiency is strictly related to how well our plasmonic structures match the excitation wavelength. There can be variations in the gold structures during the fabrication process that cause a mismatch between the predicted (785nm) and the actual wavelength at which the excitation is maximum. The larger the mismatch between the resonance frequency and the laser wavelength, the less effective the plasmon excitation would be, i.e. a lower change in conductance is observed.
Figure 3.5: Two dimensional electrical-optical scan of the sample. A) 3D representation of the current distribution (z-axis) in the x-y plane of the chip. B) Same distribution as in A, but on a 2D-plane, the colours indicate the increase in the measured current. C) Current scan along the x-axis of the relative change in conductance in percentage, calculated as the difference between the maximum and the minimum value divided by the baseline value.
4 Results and discussion

In this section the different experiments are illustrated and their outcome discussed. The experiments in the first section are related to the study of plasmonic heating and pore stability. The second part deals with DNA translocation experiments, focusing on new effects with plasmonic nanopores.

4.1 Studying plasmonic heating with nanopores measurements

As described in sections 2.2 and 2.3 and presented in reference 37 and 38, when surface plasmons are excited in gold nanoparticles, such as our bowties, the incident electromagnetic field causes an increase of the metal temperature. This increase depends on the absorption cross section of the particles, which is directly proportional to the magnitude of the incident power. The cross-section is influenced by the particle dimensions and light polarization.

Effect of incoming light polarization and bowtie dimension on plasmonic heating

As illustrated in the section on plasmonics, the absorption cross-section of a system consisting of two metallic nanoparticles shift depending on the polarization of the incoming electric field and the particles dimensions. Here we study how the plasmonic heating varies for different bowtie lengths at longitudinal and transverse polarization. The term longitudinal is referred to the major axis of the bowties. As depicted in figure 2.16, the long axis of our particles system is the one connecting the two triangles, so for us the longitudinal polarization has to be considered as the one parallel to that axis and transverse polarization is intended as the one perpendicular to it. As illustrated in figure 2.17 the simulated absorption cross-section at our laser wavelength is maximum at longitudinal polarization for particles with a length ranging from 50 to 70 nm. The situation is reversed for bigger bowties. We therefore expect to have highest heating of the particles that are larger than 80 nm for an incident electromagnetic field that is transversely polarized.

The local heating, $\Delta T$, can be quantified by exploiting the change in the nanopore conductance, $\Delta G$, according to the following formula:

$$\Delta T = \left( \frac{a}{b} + T_0 \right) \frac{\Delta I}{I} = \left( 32.9 \degree C + T_0 \right) \frac{\Delta G}{G} \quad (4.1)$$

where $a$ and $b$ are parameters determined by the approximate linear relation between the bulk conductivity of 1M KCl buffer and temperature, $T_0$ is the temperature of our lab and $G$ is the absolute conductance of the pore. The quantity $\frac{\Delta G}{G}$ is called relative conductance change and in order to assure consistency of the data between different samples and different polarizations, the values are normalised by the power of the incident laser light.

The relative change in conductance is obtained from 2D electrical scans through the focal plane of each nanopore, repeated for the longitudinal and the transverse polarization. A summary of the results is shown in figure 4.1.
Here the relative conductance change, $\frac{\Delta G}{G}$, normalised by the incident laser power, is plotted as a function of bowtie length. At longitudinal polarization the heating is maximum for 80 nm bowtie instead as for 60 nm, as it would have been expected from the fabrication design (see figure 2.17). In fact as mentioned in section 2.3, the bowties are designed with geometrical parameters (60nm length, 10 nm tips rounding, 10 nm gap and 30 nm thickness), such that their resonance frequency matches the laser wavelength for longitudinally polarised light, so we would expect maximum heating for the samples with 60 nm structures. The fact that we instead have the maximum heating for 80 nm indicates that the real bowties do not perfectly match the design. We carried out FDTD simulations changing the tips rounding to 10, 20 and 30 nm. From TEM images (see figure 4.2 for two examples) we measure the rounding to be about 20 nm. This value is not exactly constant for all the samples, due to variation in the fabrication. Figure 4.3 shows the simulated absorption cross sections of different bowties for 20 nm rounding. The maximum heating is expected for the 80 nm bowtie, as we observe in our experiments. Furthermore, higher heating is expected for longitudinal polarization, as confirmed by our measurements.

Also we expect different heating at different polarizations. According to our simulations the absorption cross section for the structures with a length ranging from 50 to 80 nm is higher for the longitudinally polarised light, while for larger bowties the situation is reversed, the absorption is greater for the
transverse polarization. In figure 4.1 we see that the difference between the relative change in conductance becomes smaller for very large bowties, but the expected higher heating for the transverse polarization is not observed. This fact is in disagreement with what is predicted by the simulations. One reason could be the fact that the polarization was not perfectly longitudinal or transverse in our setup. A small fraction of transverse light was part in the longitudinal configuration and vice versa.

Figure 4.3: Simulated absorption cross section for 60, 80 and 100 nm long bowtie with 20 nm rounding. The different colours correspond to different lengths. The full line plots are for longitudinally polarised incident light, while the dashed plots are for transverse polarization. The black dashed line correspond to the laser wavelength.
Pore stability

Upon laser illumination it was found that changes go on in the sample. Plasmonic excitation causes the pore’s structure to change, probably both enlargement and shape changes take place. The aim of this section is to understand how the resistance, and consequently the pore, varies according to the laser exposure time and power. This type of measurement gives us a double information: from the variation in the resistance value we can get an idea of how much the pore structure changes, while from the difference in resistance when the laser is switched on and off we can calculate the relative change in the pore conductance and the relative increase of the temperature according to formula 4.1.

The purpose of these experiments is to examine the stability of the pore under laser illumination, by monitoring its resistance and electrical stability. This also allows us to establish the right conditions for our following experiments.

These experiments are performed with two different samples (see figure 4.4) under similar conditions and following the experimental procedure described in sections 3.1 and 3.5. Briefly, after chip cleaning and mounting, the linearity of IV curve is checked, the pore resistance is determined and the pore co-aligned with the laser by scanning the membrane. In order to quantify the change in pore conductance and structure, we shine the laser onto the pore at intervals of 5 seconds and we acquire the value of the resistance both when the laser is on and when it is off, at different power values. To assure consistency of the data at different powers, the pore location and laser focus are checked frequently.

The change in resistance as a function of the exposure time is shown in figure 4.5. Each section corresponds to a different laser power. The data presented are the ones acquired while the laser is switched off for 5 seconds, in order to see only the effect of the pore growth and not the increase in current due to heating. For low powers (1 to 7 mW) the resistance of the pore changes very slowly indicating that the local heating is still moderate and it does not influence the pore structure. A more rapid pore enlargement begins at about 10 mW, indicating that for this power the plasmon excitation and consequently the heating is becoming large enough to affect the pore structure. The decrease in resistance means that the pore is growing in size. For very high powers (30 to 50 mW) the resistance decreases

Figure 4.4: TEM images of the samples used to investigate the stability of plasmonic nanopore. The gold nanostructures (in dark) and the nanopores are visible

The change in resistance as a function of the exposure time is shown in figure 4.5. Each section corresponds to a different laser power. The data presented are the ones acquired while the laser is switched off for 5 seconds, in order to see only the effect of the pore growth and not the increase in current due to heating. For low powers (1 to 7 mW) the resistance of the pore changes very slowly indicating that the local heating is still moderate and it does not influence the pore structure. A more rapid pore enlargement begins at about 10 mW, indicating that for this power the plasmon excitation and consequently the heating is becoming large enough to affect the pore structure. The decrease in resistance means that the pore is growing in size. For very high powers (30 to 50 mW) the resistance decreases
slower than for 10 to 25 mW range, probably due to the fact that the pore structure change significantly, the diameter is bigger and the shape is not maintained, causing the plasmonic structures to move from their initial position. This misalignment of the gold structures has a negative effect on plasmon coupling, causing less plasmons excitation.

Figure 4.5: Resistance versus time measured at zero laser power, but after 5 seconds illumination at the different laser powers.

To verify our assumption that heating plays a strong role in the structural changes of the pore, we conducted two control experiments with non-plasmonic pores. The samples were heated by boiling them in buffer solution for 10 minutes. In both cases the resistance decreased, from 18.6 to 11.1 MOhm in one case and from 17.6 to 5.8 MOhm in the other. Other effects can also affect plasmonic pores. It is for example possible that the gold reacts with the chlorine present in the buffer to form gold-chlorine compounds and that this effect is somehow enhanced/dependent on plasmonic excitation.

Another fact that is worth mentioning for the coming DNA translocation experiments is that the misalignment of the gold structures has to be avoided, because it would cause the hotspot, from the nanopore and consequently the evanescent field used for trapping, to disappear.

The observations above are strengthened by the measured relative conductance change and corresponding temperature increase at different powers. The data are summarized in figure 4.6. Here we can see that the heating, i.e. the plasmon excitation, increases linearly with power up to 7 mW. It is maximum at about 10-25 mW, corresponding to the values for which the pore resistance starts decreasing the most. For higher powers the heating becomes lower due to changes in the structure. This slows the pore growth, as observed in figure 4.5. The range of these values changes between samples, because of the fabrication process the bowties length can be slightly different from chip to chip (see figure 4.4), causing the efficiency
also of initial plasmon excitation to vary. The more efficient the plasmons excitation is the greater the shift in conductance and heating.

![Graph](image)

**Figure 4.6**: Relative conductance change and the corresponding increase in temperature as a function of the incident laser power for one of the samples.

The effects described are mainly related to the plasmonic excitation and not the laser beam itself. This conclusion can be drawn by comparing our experiment with the results presented in ref. 54. In the experimental paper Keyser at al. used a non-plasmonic pore drilled in a SiN free standing membrane to measure the three-dimensional intensity profile of a laser by monitoring the change in the ionic current. They performed the measurements with a power range that is one to two orders of magnitude higher than what we used for the plasmonic pores and they got an increase in conductance much lower than for our pores. Furthermore, they obtained a linear relation between the conductance increase of the base line and the laser power, while for our plasmonic pores a non-linear behaviour was observed, see figure 4.7 blue circles. This indicates that the structural changes of the pore upon laser illumination are more severe in our plasmonic chips compare to the non-plasmonics ones even when hit by the laser beam, due to the strong heating effects of the gold bowties. Once the laser in switched off the conductance decreases but it does not go back to the value that it had before the last measurement with laser. The data shown in red in figure 4.7 are acquired at 0 mW power, but they are plotted as a function of the power of the light striking the pore before the laser was switched off. The arrows indicate the sequence of the data acquisition. The increase in conductance for the 0 mW measurements indicates how much the pore grew upon the laser illumination.
Figure 4.7: Increase in pore conductance as a function of the incident laser power. The data represented in blue are acquired with the laser striking the sample, while the data in red after the laser is switched off. The arrows indicate the temporal acquisition of data.
4.2 DNA translocation experiments

In this section we present the results and discussion on DNA translocation experiments with plasmonic nanopores. First we focus on how to best optimise the data acquisition and data analysis parameters for our type of pores. Next we focus on plasmonic effects on conductance blockade depth. Last, the experiments for plasmonic trapping attempt of DNA are discussed.

Optimising the experimental parameters and the analysis method

In order to set the best parameters for data acquisition and analysis in DNA translocation measurements, we conduct a control experiment with a non-plasmonic pore.

The chip is prepared as described in section 3.1 and mounted on the flow cell. The pore resistance and the current trace stability are checked before adding the DNA solution to the flow cell. The DNA used in all our experiments is taken from lambda-bacteriophage. This type of DNA is commonly used in translocations experiments\cite{28,29} and it is in 48.5 kilo base pairs long. The sample is prepared by adding the electrolyte to the mixture of DNA and deionised water. The final salt concentration is 1M for experiments employing KCl buffer, while it is 2M for LiCl. The DNA concentration is kept between 10 to 15 ng/µl. Then the sample (DNA in buffer) is heated to 65°C for 10 minutes, to separate the molecules from each other, because they tend to stick together in solution.

The pore used in the control experiment has a resistance of 9.3 MΩ corresponding to a diameter of around 17 nm. We conduct DNA translocation measurements at different voltages, +50 mV and +100mV, changing low pass filter frequency and the threshold to detect events. The latter is important, because it sets the depth of the blockades recognised as events by the LabView program. The filter frequency is fundamental to minimize the noise as much as possible, enabling the detection of the small variations in current caused by the translocation of a DNA molecule.

![Figure 4.8: Schematic representation of how the histogram of current (and conductance) blockades is obtained from the raw current trace.](image)

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We are interested to measure the dwell time and the depth of the current blockades and their distribution. The histogram representing the blockades distribution is obtained by counting all the current data points of the detected events. The schematic principle is illustrated in figure 4.8. Let us assume that we measure two events; one translocation of DNA in a linear fashion and one folded event. The points of the current baseline are counted as the peak that we see at 0 nS. The points corresponding to the linear translocation and the less deep part of the folded translocation form the second peak of the histogram and the deep part of the folded events is counted as the third peak. We note that the blockades histogram is strongly influenced by the detection threshold and the filter frequency. In figure 4.9 the same set of data is shown with different analysis parameters. In one case the parameters used for the analysis do not enable us to identify different levels of the blockades, whereas peaks in the current histogram are visible when a different filter frequency and detection threshold are chosen. This is due to the fact that the detection of an event is possible only if the noise of the current signal is lower than the depth of the blockade. Instead the scatter plot of the dwell time is less influenced by those parameters, because the observed length of an event is not dependent on which frequency the current signal is filtered at. Our optimised parameters are an events detection threshold of 5 times the current standard deviation and a filter frequency of 2 kHz.

**Figure 4.9:** Comparison between data acquired with the same parameters, V= 100mV filter f=2kHz treshold=4, but analysed with different filter frequencies. A) filter frequency 5kHz and B) filter frequency of 2 kHz
**Plasmonic effects on DNA translocations**

In this section we investigate the influence of plasmonic particles on the depth of DNA translocations blockades. Based on the principle that the plasmonic resonance depends on the dimension of the metallic structure, it is possible to tune the resonance wavelength by changing the length of the bowtie antennas. We perform DNA translocation measurements for samples of different dimensions to explore how the blockades are affected by the shift in resonance of our plasmonic structures due to the presence of the DNA molecule. In fact the basic principle of plasmonic sensing is to monitor the change in the resonance curve when biological molecules are close to the metallic structure.\(^{34,36}\)

As for the DNA translocation experiment with a non-plasmonic pore, we are interested in characterise the type of events through their dwell time and current blockade distributions. First the pore is electrically characterised and localised on the membrane. Next the sample containing DNA is added. After the DNA is added we start acquiring current traces without exciting plasmons (the laser is kept off) to get a reference data set that enables us to quantify the influence of our nanoantennas in the following measurements. This measurement is carried out like a regular translocation experiment with a normal SiN nanopore: a 100 mV voltage is applied across the membrane, the current trace is monitored and the blockades caused by DNA going through the pore are recorded. Then the laser is turned on and measurements at different powers are carried out. The light polarization used in these experiments is longitudinal because we want to excite plasmons to generate the evanescent electric field hotspot in the nanopore. The laser power is changed in discrete steps in a range between 3 to 15 mW, depending on the stability of the chip, to the change the amount of plasmonic excitation. In general these values are still low enough to measure for an adequate time without causing major changes in the pore and bowties.

The results of a translocation measurement with no plasmonic excitation are shown in figure 4.10. In the scatter plot each point corresponds to an event quantified according to its dwell time and current blockade depth. It is possible to distinguish between two populations corresponding to translocations of linear and folded DNA. The folded DNA cause a deeper blockade than the one translocating in a linear fashion, because it blocks a bigger portion of the pore, so the amount of ions going through is less. The histogram represents the blockade distribution of the measurement in terms of both absolute conductance and relative conductance. The former is the difference between the baseline value (open pore) conductance and the conductance when a molecule is in the pore, so it practically corresponds to the depth of an event. The latter is the absolute conductance blockade divided by the open pore conductance. It is possible to identify three peaks, the 0 peak corresponding to the open pore value, the first to the blockade by a linear translocation and the second by a folded translocation. In principle the distance between the 0 and the second peak is double than the distance between the 0 and the first, because the portion of the pore occupied by a molecule translocating in a folded way is double than the one going through in a linear way.
In our type of experiment the outcome is not limited to one set of data, but we have multiples one corresponding to each power that the measurements are carried out at. Figure 4.11 shows an example of what we observe. What is clearly visible in the conductance blockade histogram are the shifts between the position of the peaks for different powers. The blockades seem to be deeper with increasing power. This is partly due the heating effects of the gold particles. As mentioned above, the metal heats up when it is struck by the laser light and this causes a change in the buffer conductivity. Recalling that the conductance is directly proportional to the conductivity (equation 2.1) and it increases with temperature, we expect the absolute blockades to be deeper as the heating becomes larger. This fact causes the shift in the absolute blockades histogram between the data acquired at 0 mW and the ones acquired with the laser hitting the pore. In figure 4.12 the conductance shift with respect to the measurement at 0 mW is plotted as a function of laser power. The shift increases approximately linearly with the power of the incident light. In fact, the blockade is proportional to the buffer conductance, which increases linearly with temperature. The heating is directly proportional to the power of the incident light. The heating effect on the
conductance blockade depth can therefore be eliminated from the data representation by dividing the absolute conductance of the blockades by the baseline conductance value, because both quantities are directly proportional to the buffer conductance, which increases linearly with temperature. These corrected results are shown in figure 4.11(right). Interestingly the relative blockades do not overlap. Instead the relative conductance peaks when the laser is on are shifted towards smaller values compared with the plasmons not being excited. We think this effect is related to changes in the plasmons resonance wavelength due to the presence of the DNA in the hotspot during translocations.

![Figure 4.11: Histograms of the absolute (left) and relative (right) conductance change. The peaks of the measurements at 4 and 8 mW are shifted respect to the peak at 0mW, both for the absolute and the relative conductance](image)

![Figure 4.12: Shift in the conductance peaks as a function of the incident laser power. The blue squares and the diamonds are the shift of the 1st and the 2nd histogram peak, respectively. The full lines are linear fits, with the fixed parameter of intercept at 0.](image)

Our observation is supported by the fact that the shift in the relative conductance peaks is not present in a non-plasmonic pore, as investigated by monitoring DNA translocation for that type of sample with and without laser striking the pore. The results of this measurement are shown in figure 4.13. There is a shift between the laser on and laser off data in the absolute conductance peaks, but the peaks are aligned again when we normalise with the respective baseline values. It is worth mentioning that in this experiment the
heating is due to the laser and not to gold particles. To obtain a large enough increase in temperature for a good comparison with plasmonic samples, the laser power used is 56 mW, much greater than the ones employed with plasmonic nanopores, which is of few mW.

![Figure 4.13](image)

**Figure 4.13**: Histograms of the absolute (on the left) and relative (on the right) conductance change for a non-plasmonic chip. The absolute conductance peaks of the measurement with the laser on are shifted respect to the no laser peak, while they are aligned for the relative blockade.

To better understand the possible origin of the relative conductance blockade shift we look at the plasmon excitation spectrum and think about the basic principle of plasmonic sensing. The spectral position of the plasmonic resonance changes according to the surrounding environment, including the presence of molecule on or near the metallic surface. To explain the principle, a schematic drawing is shown in figure 4.14. The black line represent the current trace and a blockade caused by a translocation of a molecule. The presence of the molecule causes a shift in the resonance curve towards longer wavelengths (red shift) because the DNA has a higher refractive index than water. If we assume that plasmon resonance curve is blue shifted relative to the laser wavelength, the red shift will cause an increase in heating. This change in temperature causes an increase in the current (green line in figure 4.14) and the event blockade is therefore smaller (delta G2) because it is measured from the base line acquired without the molecule (black line). The situation is reversed if we consider the resonance curve to be on the right of the laser wavelength. In that case the heating is expected to be less when the DNA molecule is in the pore, causing the conductance to decrease and consequently the blockade to be deeper if measured from the baseline without the molecule.
To explore this hypothesis we conduct experiments with bowties of different dimensions, ranging from 50 to 70 nm in length, to change the plasmonic resonance of the nanoantennas. When the spectrum is shifted because of the different structure, the shift in the resonance value at the laser wavelength caused by the DNA molecules is larger or smaller depending on the slope of the resonance curve. To get a better picture of the principle we can observe figure 4.15. The three colours represent the excitation spectra for bowties of different lengths. The absorption cross section of the three that has the highest value at 785 nm corresponds to the 80 nm bowties, while the one on the left is for a smaller structure. The red one is for a bigger structure. The spectra drawn with a narrow line are there to illustrate how the presence of a DNA molecule is shifting the respective curves. The results are shown in figure 4.16. For all the structures the same behaviour is verified, but many variations are found between samples with the same designed structure. This is due to multiple factors related to the imperfection of the fabrication process at the nanoscale. Although the bowties are designed to be identical, the gap between the triangles can change, as well as the actual length of the particle and the curvature of the tips.

**Figure 4.14:** Illustrative sketch of the shift in the conductance blockades caused by the change in the resonance curve due to the presence of the DNA molecule in proximity of the gold structures.

**Figure 4.15:** Simulated absorption cross sections (bold line) and the exaggerated shifts due to the presence of the molecule in the pore for 20 nm bowtie rounding. The black arrows correspond to the shift that we expect to measure in the relative conductance. The arrow pointing up corresponds to a shift towards lower values of conductance blockade (as represented in figure 4.14). The arrows pointing down correspond to a deeper conductance blockade.
Figure 4.16 present the central results of these experiments. Here we plot the shift of the relative conductance peak of DNA translocations as a function of the bowtie length. The shift increases with the bowtie length, but it is really hard to determine if the behaviour is linear or not due to intrinsic variations in the bowties dimensions. For all the structures, we report the data of, the maximum of resonance curve is on the left side of the laser wavelength. Following the principle illustrated above we expect the shifts to be negative, i.e. the shift of the first and second peak in the relative blockade histogram is on the right of the 0 mW measurement histogram. When the DNA molecules go through the pore they cause an increase in heating, consequently the current is shifted upwards resulting in a smaller blockade. This effect is expected to become smaller as the maximum of the resonance curves approaches the laser wavelength and become positive (deeper blockades caused by less heating) for bowties larger than 80 nm. Unfortunately the experiments with such big structures present some additional complications, such as instability of the pores and DNA molecules sticking to the gold. We are currently working on a better understanding of the problem and possible solutions for it.

![Graph showing the shift of the relative conductance peak as a function of bowtie length](image)

*Figure 4.16: Relative conductance's peak shift of the 1st peak of the histogram normalised by the incident laser powers as a function of bowtie length. The error bars correspond to the standard deviation of measurements at different powers for the same chip.*

The change in the plasmons resonance might be not the only effect causing the shifts of the conductance blockades. An alternative effect is related to thermophoretic forces arising from the temperature gradient in the proximity of the pore, created by the highly localised heating. Molecules tend to move from hot to cold regions. It was theoretically demonstrated by molecular dynamics (MD) simulations that a ssDNA going through a locally heated pore feels the thermophoretic force on the ends located right outside the pore. The heating is localised in the immediate surroundings of the pore (~10 nm), so the portion of the polymer translocating is pulled by the thermophoretic force from inside (hot area) to outside (cold area) the pore. The pulling is exerted on the part of the DNA that just translocated and on the one that is about to, causing the portion of DNA inside the pore to stretch. The stretching results in fewer nucleotides present in the pore, i.e. the portion of the pore occupied by the polymer is smaller than in a non-heated
sample. The current blockade is consequently expected to be less deep. This effect could contribute to the relative conductance peaks shift observed with our plasmonic pores. Furthermore we note that the unwinding of the polymer represents an advantage for the single-base measurements, because it allows the reading of the nucleotides one by one, assuming the time to collect the signal from a single base was enough. Even though the reported simulations were performed for ssDNA, the thermophoretic effect is worth to be considered in explaining the outcome of our measurements.

**DNA trapping in plasmonic pores**

The purpose of this section is to explore the ability of the plasmonic field to trap DNA molecules that translocate through the pore. The process is governed primarily by two competing forces: the electrophoretic force driving the DNA from the cis to the trans chamber and the force felt by the molecule in the proximity of the plasmonic electric field.

According to preliminary MD simulations we expect to achieve a trapping force high enough to overcome the electrophoretic force when we excite plasmons with an incoming light power in the order of ten mW. This might represent an issue for our plasmonic samples because according to the heating experiments that is the value around which major changes starts happening in the pore structure due to the increase in local temperature. Therefore we decide to exploit the fact that the electrophoretic force can be controlled by changing the number and the type of counter ions that bind to the DNA backbone. In order to reduce the electrophoretic force we here use a lithium chloride buffer at 2M concentration instead of KCl at 1M. The Li+ ions binds more strongly to the DNA compared to the K+ ions and their number is greater for higher concentrations.²¹ By comparing the dwell time of experiments carried out in the two buffers without laser excitation, it is clear that the LiCl effectively reduces the electrophoretic force, as shown in figure 4.17.

![Figure 4.17: Scatter plot of the detected events with two different buffers. The data were acquired with different pores with resistance value around 12 MΩ at 0 mW. Longer translocation times are observed for LiCl buffer as expected.](image)

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If the plasmonic forces are strong enough to compensate the electrophoretic drag, we expect to observe a longer dwell time when the plasmons are excited than when they are not. The increase in the translocation time is also expected to be linearly dependent on the power of the exciting electric field.

The samples used in these experiments are the ones with a designed bowtie length of 60 nm and the DNA is added from the membrane side instead of the pyramid side. This choice is motivated by the fact that the polymer will experience the plasmonic forces right before entering the pore. If it is trapped by the optical fields, then the part dragged by the electrophoretic force will be stalled inside the pore.

![Figure 4.18: Current trace acquired without plasmonic excitation. The current is negative and the blockades are upwards because the DNA was inserted from the membrane side and the applied voltage is negative. Buffer conditions LiCl 2M.](image)

Figure 4.18 shows the regular DNA translocation events at zero power. At low powers the only effect of plasmons is the one studied in the previous section, i.e. a shift in the relative conductance depending on the bowties structure and incident laser power. The interesting observation is that increasing the power of the incoming light there is threshold at which very long and multiple blockades start appearing. The observation of a laser power threshold at which the multiple blockades start happening was repeatable for multiple samples. An example of these events is shown in figure 4.19. Here we can see the recorded current traces and DNA translocation events for no plasmonic excitation and for excitation laser light with a power of 12mW. We clearly see that in the second case the time scale of the blockades is 1 to 2 orders of magnitude greater than for regular events. Furthermore, the steps in the current are on regular and repeated values. A way to have a quantitative representation of these multiple events is to plot a histogram similar to what is done for the normal translocations blockades. Such plot is shown in figure 4.20. This plot is obtained similarly to the current blockades one (see figure 4.8 for example), but instead of counting only 50 point before and after translocation events, the whole trace is considered, due to the presence of very long (several seconds) blockades. We can observe the presence of discrete steps in the histogram, indicating discrete levels in the current, corresponding to different number of molecules inside the pore. A realistic explanation is that the molecules are in fact attracted to the bowties by the plasmonic electric field gradient. More than one can get trapped in a progressive way (multiple steps towards a lower current magnitude), released and re-trapped independently. The fact that one or more molecules are already
trapped does not seem to prevent the trapping of others. When the laser is switched off, the multi-level long blockades are not visible anymore, but we still see single long blockades. These events can be caused by a DNA molecule sticking to the gold or to the SiN.

Figure 4.19: Current traces A) Trace recorded at a laser incident power of 10mW, it is clearly visible that something is getting trapped in the pore. B) Zoom in to A to see the multi level steps. C) Trace acquired at 0mW, after the measurements with the laser on.

Figure 4.20: Current blockades histogram of long and multiple steps events. The peak corresponding to the baseline is the one around -17 nA.
In order to avoid sticking one can coat the gold structure with 6-mercaptop-1-hexanol (MCH) molecules and thiol-PEG molecules that binds covalently to the metal. Once the gold is coated with these molecules the DNA molecules should not bind to the gold. So far we have encountered some issues with coating the gold particle: with the heating generated by the plasmonic excitation the molecules tend to de-attach from the metal. In figure 4.21 a 2D electrical scan of a coated sample is shown. The current changes in a progressive way, from higher to lower values, when the laser comes close to the pore. A likely explanation is that the heating of the gold particles caused some coating molecule to de-attach and then they partially block the pore for while. The resistance eventually goes back to the open pore value, indicating that the pore got gradually free again and that the molecules diffused away with the ions flow through the pore. The coating procedure will be optimised by testing the effectiveness in preventing DNA sticking and check if the molecules that de-attach are the ones covalently bound to the gold or some molecules weakly attached to the SiN membrane. In order to test this assumption, we plan to fabricate a very flat gold surface, coat it with thiol-PEG molecules, add some DNA molecules and check if they bind to the gold by scanning the surface with atomic force microscope (AFM).

Figure 4.21: 2D electrical scans around the pore for gold coated with MCH molecules.

Further measurements have to be performed concerning the trapping. It is fundamental to confirm that what we call optical trapping is due the evanescent electric field in the hotspot and not caused by chemical modification of the gold due to the high heating. It is plausible that above certain heating threshold the gold starts interacting with the ions in the buffer. This fact could make sticking of DNA molecules more likely to happen. The effect of heating can be verified independently from optical trapping by illuminating the sample with transverse polarised laser beam. As mentioned above, this causes heating of the structure without exciting the hotspot. If it is verified that the evanescent optical field is responsible for the observation of long translocation events, the trapping time needs to be studied as a function of incident laser power. It may also useful to reduce the DNA concentration, so it is possible to have one or very few molecules in the pore at a time. It is also important to check that the effect does not persist once the laser is switched off and that translocation events happen in a regular way. By alternating the incident optical field it would be possible to repetitively monitor the events under the two different conditions, keeping the other experimental parameters constant.
Plasmonic effects on translocation events frequency

In our experiments on DNA translocation through plasmonic pores, we observe an enhancement of the events frequency that depends on the incident laser power. In figure 4.22 the ionic current time series at conditions of no plasmonic excitation (blue trace) and for plasmonic heating due to laser illumination (red trace) are shown. The increase in the event rate is clearly visible. In table 4.1 we report the calculated event frequency for different incident laser powers.

![Current traces and translocation events in 2M LiCl buffer with the laser off and striking the pore at 7.5 mW](image)

**Figure 4.22: Current traces and translocation events in 2M LiCl buffer with the laser off and striking the pore at 7.5 mW**

A similar frequency enhancement effect was reported in a recent papers by Reiner et al.\(^5\) They combined an *alpha-hemolysin* nanopore with three gold nanoparticles and study the temperature effect on ionic current and on the translocation of a polyethylene glycol (PEG) molecule. The observe, among other effects, an increase of events rate when going from room temperature (21° C) to 54° C. Their hypothesis is that the enhanced capture rate is due to the decrease in solvent viscosity, structural changes in the polymer and thermophoresis. To get a better picture of the how the DNA molecules go from the solution into the pore, we have to consider the capture radius and the capture rate of the molecules.\(^6\) The capture radius, \(r^*\), is the parameter describing the area around the pore where the molecules feel the effect of the dragging force that drives them into the pore. The capture rate, \(R_c\), is the parameter that quantifies how many molecules are captured by the pore and in a diffusion limited regime is expressed as:\(^5\)\(^6\)

\[
R_c = 2\pi D r^* \tag{4.2}
\]

Where \(D\) is the diffusion coefficient of the DNA.

Regarding the electrophoretic force acting on the DNA, the capture radius is inversely proportional to the diffusion coefficient, so the dependence from \(D\) is eliminated in the capture rate equation.\(^5\)\(^6\) The rate still depends linearly on the electrophoretic mobility of the molecule which is inversely proportional to the solvent viscosity. Hence, \(R_c\) is expected to increase when decreasing the viscosity of the buffer. It is well known that the viscosity, \(\eta\), of a liquid depends on its temperature, \(T\), according to\(^5\)\(^7\)
\[ \frac{1}{\eta} = a_0 + a_1 T + a_2 T^2 + a_3 T^3, \]
where \( a_i \) are the parameters for polynomial depending on the liquid considered. Therefore for an increase of temperature due to plasmonic heating the capture rate is expected to increase.

A similar approach can be used in considering possible thermophoretic forces due to temperature gradients. In this case the capture radius does not depend on the diffusion coefficient, \(^4\) so, knowing that \( D \) is directly proportional to the temperature (Einstein relation), \(^5\) the increase of capture rate can be deduced directly from the above equation.

<table>
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<th>Laser P (mW)</th>
<th>Event frequency (1/s)</th>
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</thead>
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<tr>
<td>0</td>
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</tr>
<tr>
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<td>1.17</td>
</tr>
<tr>
<td>0</td>
<td>0.09</td>
</tr>
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<td>1.76</td>
</tr>
<tr>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td>12.5</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Table 4.1: Events frequency at different laser powers in LiCl buffer
5 Conclusion and outlook

In this work we characterised the optical and thermal response of plasmonic nanopores and we explored the new effects and sensing possibilities offered by this new device. We showed that this new combination can serve multiple purposes. Interesting observations in DNA translocation arose not only from the presence of a strong and confined electric field in the hotspot, but also from thermal effect. In fact plasmonic structures represent a practical realization of a highly localised and tuneable source of heat that can be used to explore thermal effects in solid state nanopores and thermophoretic forces in DNA translocation processes.

Regarding the optical characterization, our results do not completely agree with what is predicted by the simulations. The predicted trend is verified, but for structures larger than 80 nm, we do not see higher heating for transversely polarised incident light respect to the longitudinal polarization. This may be due to the light at the sample having both the components. The longitudinal component is larger than the transverse in one case and vice versa. Further measurements with the corrected polarization have to be performed in order to verify the agreement between the properties of the real structures and the behaviour predicted by the simulations.

Through the studies on the pore stability we were able to establish a range of laser powers at which our device is stable. The response changes from sample to sample, primarily due to differences in the plasmonic structures and in the pore. Special attention needs to be paid when performing experiments with big (80nm) bowties since the plasmonic excitation is greater than for smaller structures. For example the laser power used to localise the pore on the membrane has to be keep as low as possible to avoid damage. The stability of the device could also be influenced by the chlorine ions present in the buffer reacting with the gold. This issue needs to be further examined by employing other kinds of buffers that do not contain chlorine.

In DNA translocation experiments we observed a variety of new and interesting effects arising from the combination of plasmonics with solid state nanopores. One of the most interesting challenges is to separate the effects due to the very high and localised heating and the effects due to the change in plasmonic resonance in translocation measurements. An individual quantification of these two effects is needed to draw reliable conclusions from our observations. A way to independently study the effect of plasmonic resonance and thermophoresis can be achieved by considering that the plasmonic resonance shift is greater for longitudinally polarised incident electromagnetic field. As described above, this polarization generates the antenna’s hotspot, which resonance wavelength is strongly influenced by the presence of a molecule in its proximity. The effect is combined with generation of heating accordingly to the absorption cross section. Instead for transverse polarised light, the shift in plasmon resonance is a minor effect compared to heating. So it may be possible to evaluate the heating effect alone by measuring DNA translocation under illumination of the sample with a transversely polarised laser beam. Then the same measurement can be repeated for another chip under longitudinal polarization by adjusting the laser power to get the same amount of heating obtained with the previous sample. In this second case also the plasmonic resonance shift effect is present. By comparing the outcome of these two experiments it is in principle possible to isolate the effect of the two phenomena and establish which has the stronger influence.
Measurements at different polarizations can also be useful to improve our trapping experiments. As mentioned in the discussion, it is important to confirm that the trapping is due to the electric field in the hotspot and not to other factors such as sticking of DNA to the gold structures. The tendency of the molecules to stick to the metal could be enhanced by chemical modification induced by high heating. So conducting a DNA translocation experiment having plasmonic heating without exciting the hotspot can provide a better understanding of the potential effect of gold chemical modification. As mentioned previously these conditions are satisfied when the sample is illuminated by light having a transverse polarization. Furthermore reducing the DNA concentration and a suitable coating of the gold will help to prevent sticking of molecules.

Once these issues are solved, more comprehensive experiments can be performed to monitor trapping. The dependence of the events from the laser power needs to be extensively studied. The ability of the optical trap to capture and release molecules can be explored by alternating the optical field. In fact, in absence of laser excitation the trapped molecule is expected to feel only the electrical force and be pulled away from the pore.

Further experiments on the enhancement of the event frequency due to plasmonic excitation will be planned. First of all control experiments with a non-plasmonic pores are necessary to investigate if the effect is due to the high temperature induced by the laser in the buffer or to the high temperature gradients induced by the plasmonic local heating. Then a comparison between the two buffers can clarify the contribution given by different thermal conductivities.

In summary, this work shows that the combination of plasmonic particles with solid state nanopores is a promising tool for new possibilities in DNA sensing and control of translocation motion. By making use of the optical and thermal properties offered by plasmonics this device opens up a new prospective in the study of DNA with solid state nanopores. Moreover, once a good understanding of their mechanism is achieved, both the optical and thermophoretic forces can be utilised to control the motion of a single DNA molecule inside the pore without the need of additional labelling. A combination of plasmonic sensing and motion control of a single molecule will also enable the study of local structures along the DNA chain.
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