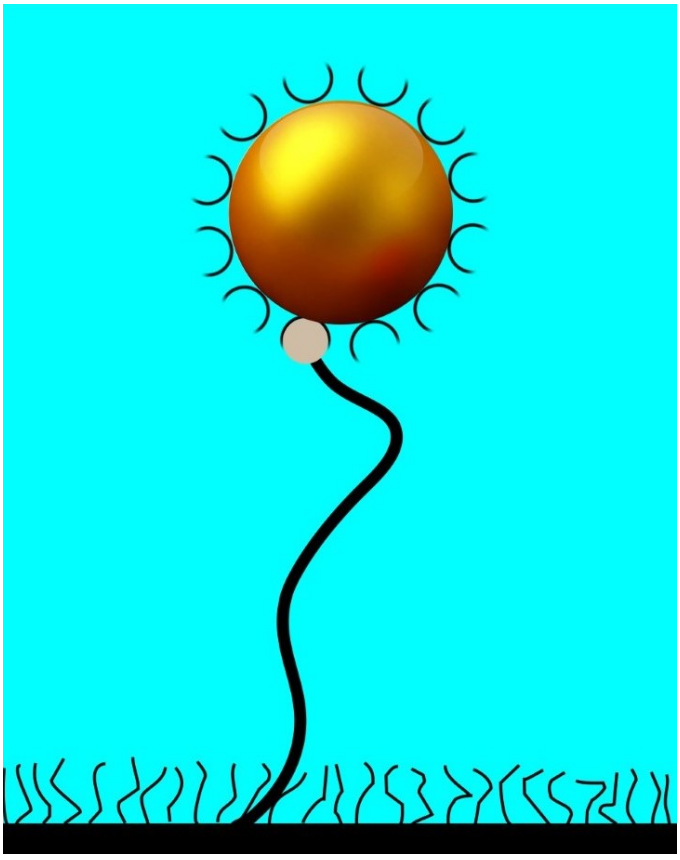
Universiteit Utrecht

Opleiding Natuur- en Sterrenkunde

Optimizing Nano-Oscillators

Bachelor Thesis

Elise Alkemade



Supervisor:

Dr.ir. Sanli Faez
Debye Institute for Nanomaterials Science

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Abstract

Measuring charges of single molecules is a difficult task. Especially small molecules in an aqueous environment are hard to characterize. One method to do this uses nano-oscillators, which are gold particles attached to a PEG molecule via a biotin-streptavidin complex. In this thesis, these nano-oscillators will be constructed and optimized, after which a potential will be applied to the system, which causes the particles to move. The observation of this movement will be carried out with a total internal reflection microscope. We find that to construct the optimal slides, the ratio of biotin to non-biotin PEG molecules should be 1:1000, and the salt concentration of the gold particle solution should be 20 mM. Further research can be done on the effects of the applied voltage, and the software used for analysis can be further refined.

The picture on the front page is a schematic image of a nano-oscillator. The half-circles represent the streptavidin molecule, which attaches to the pink biotin on the PEG molecule.

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1 Introduction

One of the challenges that occurs on the intersection of the fields of physics, biology and chemistry is studying the behaviour of single molecules on a detailed level. By studying the charge of single molecules chemical reactions affecting biomolecules can be monitored precisely. This can help in better understanding processes that take place in cells, for example[1]. Before, measuring charges has been possible mostly on a larger scale, for several or many molecules at the same time. In recent years, methods with a higher sensitivity have been developed, such as optical tweezers or scanning probe methods[2, 3]. However, these methods have their limitations, which prevent them from being able to measure single charges on nano-sized molecules[4]. A promising method that is able to do this uses nano-oscillators.

Nano-oscillators consist of a gold particles with a diameter of a few dozen nanometers, attached to a slide with a tether molecule. The tether is an important part, because it keeps the particle in place while still allowing it to move to a certain extent. This way, the particle can react to its surroundings, while not drifting away immediately. The particles' surroundings will mostly change because a potential can be placed over them. By measuring the way the particles oscillate with the potential, their charge can be determined. If their charge changes, the particles' behaviour will immediately change as well. Therefore, these nano-oscillators offer a very good technique for understanding charges and reactions at a single molecule level.

A few years ago, such nano-oscillators were constructed successfully, and they were used to measure the charge of single molecules [5]. In this thesis, the focus will be on reproducing this experiment, while using the microscopy setup present at the nanophotonics lab at UU. This microscope is especially useful for this type of experiment, as will be described later.

For reproduction, several parameters need to be optimized. The most important of those is the amount of nano-oscillators which are added to the sample, and the salt concentration of the liquid in which the gold particles are suspended. This will be tested on glass slides. After these parameters are tested, the final goal is to repeat the experiment on conductive slides, and add a potential to it. If everything works out, we will be able to see the particle move together with the AC current that moves through it. This proves that the nano-oscillators have been reproduced.

2 Theoretical background

2.1 Brownian Motion

Brownian motion is the random movement of particles which are suspended in a liquid or gas. It was discovered in 1827 by Robert Brown, when he studied pollen under a microscope[6]. He noticed that the particles were moving randomly in any direction, but could not accurately explain the phenomenon yet. This was later done by Einstein, and Brownian motion was used as proof that molecules and atoms exist[7]. When particles are suspended in a liquid which is in equilibrium, the molecules in the liquid will bump into the brownian particles. Statistically, this results in random movement.

A concept which is important with regards to Brownian motion is diffusivity, or D, which describes the distribution of particles in a solution. It can be obtained by working through several equations. The first step is solving the following differential equation for the density of an ensemble of particles ρ at point x at time t:

$$\frac{\partial \rho}{\partial t} = D\frac{\partial^2 \rho}{\partial x^2} \tag{1}$$

where D is the diffusivity we want to obtain. Solving this equation gives:

$$\rho(x,t) = \frac{N}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \tag{2}$$

which is a normal distribution. This results in a movement of $x^2 = 2Dt$ in one dimension.

The gold particles we are using during the experiments for this thesis will be subject to Brownian motion too. However, our particles are not moving freely but are instead attached to a spring molecule. Because of this, their movement is influenced by the spring constant of the tether molecule. This changes their movement slightly, but the principle of Brownian motion is still relevant.

2.2 EDL

The electric double layer, or EDL, is a layer of positive and negative charges that forms at the interface of an electrode and an electrolyte when they come into contact[8]. These can take all sorts of forms, but in the context of this experiment the electrode is a gold nano-particle and the electrolyte is the solution in which the particles are suspended. This solution has a certain amount of salt in it to make it conduct electricity.

There are several relevant components to an electric double layer, which are visible in figure 1. The innermost layer, which is inside the particle, is the surface charge. This layer consists of ions near the edge of the particle. In the picture, these are negative. The second layer is the Stern layer, which consists of positive counter-ions from the electrolyte surrounding the particle. These ions are closely bound to the particle layer, and are not influenced by electrostatic forces. From there, the diffuse layer of the EDL

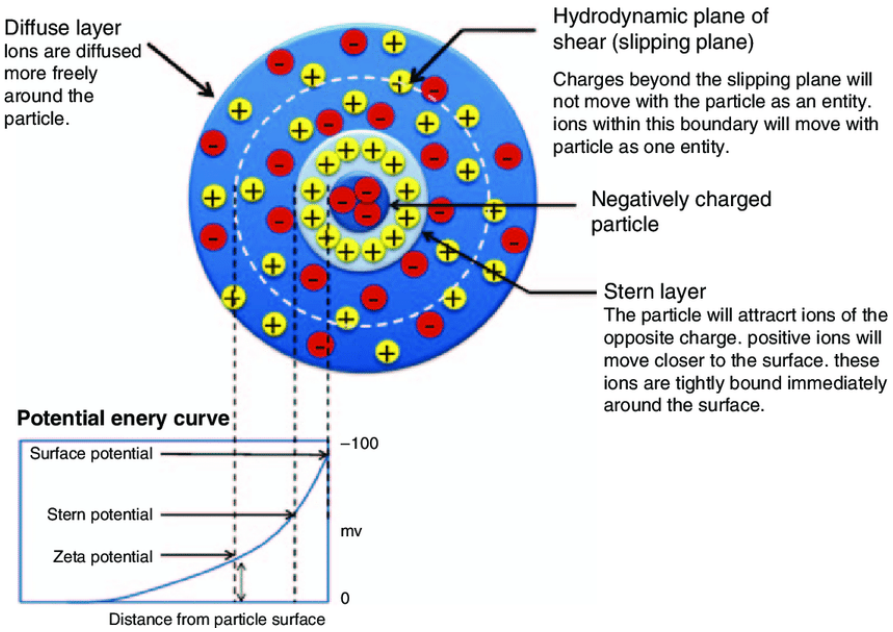


Figure 1: The electric double layer on a colloid according to the Stern-Guoy-Chapman model[9]

starts. It consists of all the ions which are outside the Stern layer, but not yet part of the neutral bulk solution. Here, Coulomb forces play a significant role. Somewhere inside the diffuse layer is the slipping plane, which surrounds all the ions which move with the colloid as it drifts through the electrolyte. The potential on this slipping plane is called the zeta potential, or ζ, which is a relevant quantity in many equations concerning the EDL.

Another relevant quantity is the Debye length, which is the total thickness of the EDL. The Debye length reaches from the surface of the particle to the edge of the diffuse layer. In total, the combination the charge of the entire EDL and with the surface charge is neutral. The formula for the Debye length is:

$$\lambda_D = \sqrt{\frac{\epsilon_0 \epsilon_r k_B T}{2 N_A e^2 I}}, \tag{3}$$

where ϵ_0 is the permittivity of free space, ϵ_r is the dielectric constant, k_B is Boltzmann's constant, T is temperature, N_A is Avogadro's number, e is the elementary charge and I is the ionic strength of the electrolyte [10].

2.3 Electrophoresis

Electrophoresis is the motion of charged particles through a fluid, under the influence of an electric field. There are several forces which play a role in electrophoresis, which will be described below[8, 11]. The first force is the electrostatic force, produced by the electric field which is causing the movement of the particles. It is a type of Coulomb

force. Secondly, there is the friction caused by the viscosity of the fluid. Finally, there is the electrophoretic retardation force. This force results from the movement of the particles surrounded by an EDL through the electrolyte. There is also the relaxation force which results from coinciding charge centers, but this can be ignored in most models.

Within electrophoresis, the quantity which is often useful to determine is the electrophoretic mobility, or μ_e, defined as $\mu_e = \frac{\mathbf{v}}{\mathbf{E}}$. This quantity has different approximations depending on the thickness of the EDL compared to the size of the particle. For a thin EDL, the Helmholtz-Smoluchowski law is valid, which is given by[12]:

$$\mu_e = \frac{e_r e_0 \zeta}{\eta} \tag{4}$$

where ζ is the zeta potential discussed above, and η is the viscosity of the fluid. For a thick EDL, the Hückel-Onsager law is applicable, and this is given by[13]:

$$\frac{2e_r e_0 \zeta}{3\eta} \tag{5}$$

2.4 Rayleigh scattering

In this experiment, the wavelength of the laser used in the microscope is much bigger than the size of the studied particles. Because of this, Rayleigh scattering occurs. Rayleigh scattering is a phenomenon which was discovered in the nineteenth century by Lord Rayleigh. It is most famously used to explain why the sky is blue, but has many more applications[14]. Rayleigh scattering is valid when the size of the scattering particle, defined as $x = \frac{2\pi r}{\lambda}$, with r the radius of the particle and λ the wavelength of the light, is much smaller than one. In our experiment this is the case, as the radius of the particles is about 20 nm and the wavelength of the laser is 488 nm.

The following formula describes the intensity of the scattered light in the regime where Rayleigh scattering occurs[15]:

$$I = I_0 \frac{1 + cos^2\theta}{2R^2} \left(\frac{2\pi}{\lambda}\right)^4 \left(\frac{n^2 - 1}{n^2 + 2}\right)^2 \left(\frac{d}{2}\right)^6 \tag{6}$$

Here, I_0 is the intensity of the incoming light, θ is the scattering angle, λ is the wavelength of the light, R is the distance to the particle, n is the breaking index and d the diameter of the particle. In our microscope setup, an evanescent wave, described in the next section, will be the incident field. As evanescent waves decrease exponentially with distance, I_0 will decrease exponentially here.

2.5 Evanescent waves

Another piece of theory that is relevant in this experiment concerns evanescent waves. An evanescent wave forms when total internal reflection occurs on the boundary between

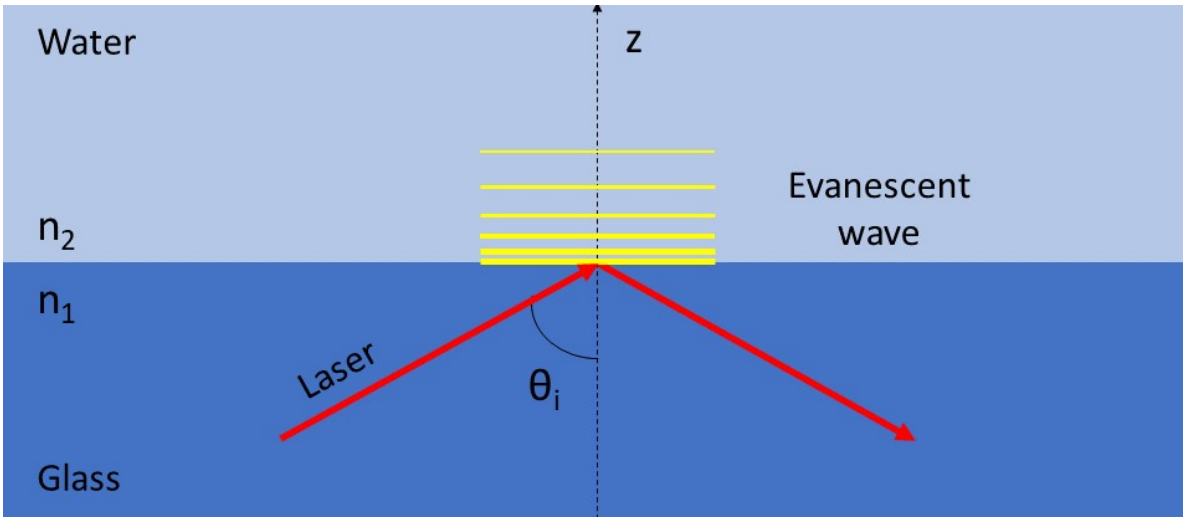


Figure 2: The formation of an evanescent wave due to total internal reflection on the interface between two materials.

two surfaces. As is known, total internal reflection can arise when a light beam travels from a dense to a less dense medium. If the angle of incidence is large enough, all the incident will reflect on the boundary, and none of it will travel to the second medium. Instead, an evanescent wave forms on this boundary. If the refractive indices of the media are denoted by n_1 and n_2, the conditions for total internal reflection are reached if the angle of incidence is larger than the critical angle, which is defined by $\theta_c = arcsin\left(\frac{n_2}{n_1}\right)$. The way in which an evanescent wave forms is shown in figure 2.

A characteristic of evanescent waves is that they decay exponentially. The wave, or field, is strongest at the boundary, and decays as it penetrates deeper into the medium. This penetration depth, or d, is an interesting quantity to study. It depends on the refractive indices of both materials, and on the angle θ of the incident light. It is described by the following formula [16]:

$$d = \frac{\lambda}{4\pi\sqrt{n_1^2 sin^2\theta - n_2^2}}, \tag{7}$$

This quantity can then be used to calculate the intensity of the field at a certain distance z above the boundary between the two materials. The intensity is calculated as follows[16]:

$$I(z) = I_0 e^{\frac{-z}{d}}, \tag{8}$$

Here, d is the penetration depth as above, and I_0 is the intensity of the field at the boundary. Here, it becomes clear that the field decays exponentially.

3 Setup

The setup used in this experiment consists of two parts. Firstly, a space is needed to prepare the nano-oscillator slides, and secondly a microscope is used to look at them.

3.1 Materials

The materials which are needed to prepare the microscope slides are listed below. Some of them are lab equipment, while others are specific to the experiment. The materials are:

- Plasma cleaner
- Plastic slide holders which fit 24x40 mm slides
- Oven
- Pipettes for 1-1000 μl
- Nitrogen stream
- Glass or ITO slides of 24x40 mm, thickness #1
- mPEG-silane-1000
- Biotin-PEG-Silane-5000
- DI water
- ethanol

With these, the construction of the slides as explained in the method, section 4, can mostly be performed. Apart from this, we need a microscope, which will be discussed below.

3.2 Microscope

The microscopy plays a crucial role in this experiment, and the specific technique is especially useful for an experiment involving colloids surrounded by an electric double layer.

3.2.1 Dark-Field microscopy

The microscopy technique used in this experiment is a form of dark field microscopy. As opposed to a 'standard' bright-field microscope, which directly illuminates the sample, a dark field microscope has a light source that does not reach the camera directly. Because of this, only the light that is reflected off the sample is visible on the screen. This gives an image in which the sample appears white against a dark background, instead of the other way around[17].

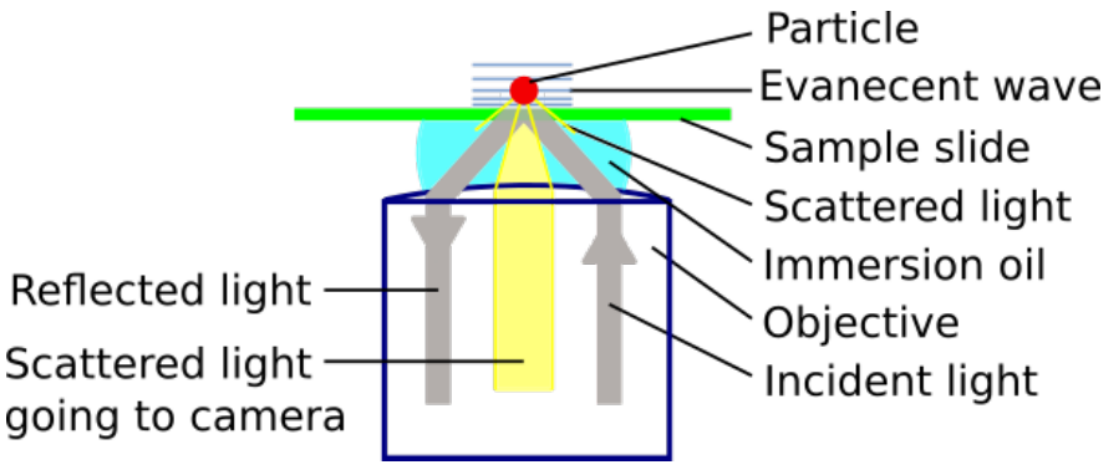


Figure 3: Total Internal Reflection Microscope[17]

3.2.2 Total internal reflection micoscropy

Specifically, the microscope used here is a total internal reflection microscope. This method is most easily explained using a picture.

In figure 3 the path of the light is clearly visible. It travels from a laser through a lens, which directs it towards the sample. The sample is positioned in such a way that all of the light reflects, and none of it falls into the camera. During this reflection, an evanescent wave is created which illuminates a small space just above the sample slide. This evanescent wave is then scattered by the particles on the slide, and the scattered light is directed towards the camera.

As shown in equation 8, the evanescent wave decays exponentially. For our experiment, the media through which the light travels are glass and water, with refractive indices $n1 = 1.52$ and $n_2 = 1.33$ respectively. This gives a critical angle of about 61°. We assume the actual angle of the laser is similar to this, about 62°. The wavelength of the laser is 488 nm. Together, this gives a value of $d = 216$ nm.

As will be explained later, the particles in this experiment will move in the z direction over a distance of several tens of nanometers. Lets take 30 nm as an example distance. Filling this in in equation 8 gives a value for $I(30) = 0.870I_0$. This means the intensity of the particle at its highest point is 0.87 times as big as the particle at its lowest. This gives a reference for the values we expect when looking through the microscope.

4 Method

4.1 Constructing slides

The goal of the experiments for this thesis is to track electrophoretic motions of nanoparticles. In this case, we use gold nanoparticles with a size of 40 nm. To keep the particles in place, they are attached to a polyethylene glycol (PEG) molecule with a length of 30 nm which acts like a spring. These molecules are polymers of the shape H-(O-CH_2-CH_2)$_n$-OH. To attach the gold to the PEG, a streptavidin-biotin complex is used. The biotin has a high affinity for the streptavidin, so the two molecules will bind easily. The gold is coated in streptavidin protein, while a biotin head is attached to the PEG. On the other side of the PEG, a silane group is added, so the molecule can adhere to the microscope slides.

It is important to note that not every molecule on the slide should have a gold particle attached to it. This would make the slide way too crowded, and the gold would not be able to move independently anymore. Therefore, the biotin headed PEGs are mixed with non-biotin PEG molecules, which act as a filler. Part of the experiment is figuring out what the ratio between the biotin-PEG and non-biotin-PEG should be.

To test this ratio, and other variables, some test measurements are done on glass slides. Later, the experiment will be repeated on a conducting material, and a potential will be placed over the particles.

The construction of the nano-oscillator slides consists of several steps. First the slides are coated with PEG, then they are made into a flowcell and finally a streptavidin-gold solution is added to them. The detailed steps are listed here.

1. Dissolve a small amount of PEG molecules in 95% ethanol, a few mg is enough.
2. Mix the solutions with biotin and non-biotin PEG so that the desired ratio is achieved.
3. Clean the glass slides in the plasma cleaner for 10 minutes
4. Add 50 μl of the PEG solution to a glass slide and sandwich another slide on top
5. Bake the slides in a 50 degree oven for 45 minutes
6. Write a mirrored L on the top right corner of both the slides while they come out of the oven. This helps to recognise the coated sides of the glass, as the L will then be in the top left corner.
7. Place the slides in a columbia jar filled with DI water to enable separation
8. Separate the slides
9. Dry the slides under a nitrogen stream.

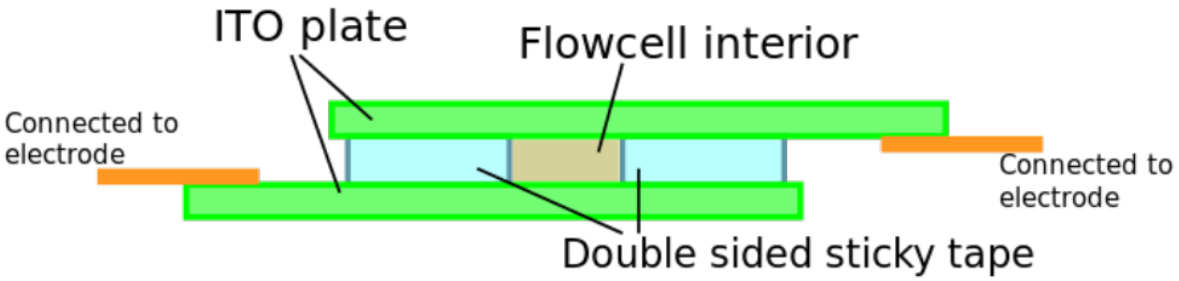


Figure 4: Side view of an ITO flowcell. The electricity is conducted through the ITO plates and the solution in the flowcell interior. [17]

After these steps, the slides are ready to be made into a flow cell. This is done by placing two layers of sticky tape onto the sample and adding a small coverslide on top. This creates a channel into which the gold solution can be added later. After all this, the slides are ready to be looked at under the microscope.

4.2 ITO slides

Ultimately, the goal of this experiment is to apply a voltage to the cell, in order to see the particles react to it. This cannot be done on glass slides, as glass is an insulator. Instead, a similar slide needs to be constructed of a conducting material. For this, we use ITO, or Indium Tin Oxide. This material is very useful for our purposes, because it is transparent for visible light, if in a thin enough layer. It also conducts electricity very well, and is resistant to wear[17]. A slight downside is that it is quite expensive, so I have only used it for my final round of experiments.

The first step in building an ITO cell is determining the conducting side of the plate. This is easily done with a multimeter, and needs to be labeled by breaking off a small corner of the slide. To clean the slides, the plasma cleaner is used again. We make sure to clean them for a shorter time, to avoid cleaning the ITO layer off. In our experience one minute of cleaning time was sufficient. After this time, the slides were still conductive, while a water droplet spread out nicely. This indicates that the slides are clean.

Next, the PEG coating can be added. This is done in the same way as for the glass slides, as described above. Finally, the flow cell needs to be made. This time, this process is more complicated than for the glass, because electrodes need to be added to the slide. The final construction is shown in figure 4. The bottom ITO plate is coated with the PEG, and the top layer is ITO plastic, as this does not need to be coated. The orange lines represent copper tape, which connects to the electrodes. These then go to a function generator which applies a voltage to the sample. At the same time, the voltage could be measured and monitored, to compare it with the movement of the particles. Due to time constraints, though, we were not able to do so yet.

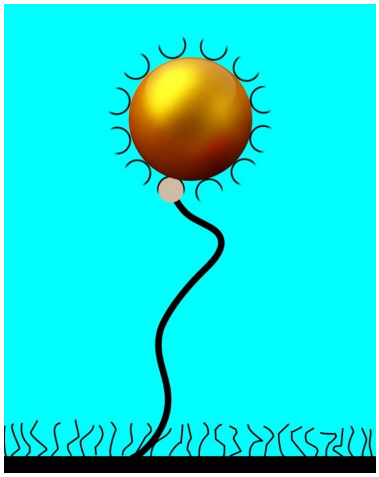


Figure 5: A schematic of a nano-oscillator slide. The pink ball represents the biotin, and the half circles coating the gold particle are streptavidin.

4.3 Finalizing slides

To make the slides ready for observation, the gold needs to be added to them. A solution of streptavidin gold in a buffer is pipetted into the flowcell. Usually, about 10 to 30 μl is enough to fill it up. With the ITO plates, extra care needs to be taken to ensure that the copper tape does not get into contact with any liquid. After a few minutes, the flow cell can be flushed with DI water to remove any unattached particles. Then the slides are ready for observation.

In figure 5 a schematic drawing of a nano-oscillator is shown. The gold nano-particle is attached to a PEG molecule via the biotin-streptavidin complex. On the bottom, the spacer PEGs, without a biotin head, are drawn.

4.4 Using the microscope

4.4.1 Observing slides

To finally observe the slides, they need to be placed under the microscope. The first step is to add a small amount of immersion oil to the objective. Next, the slide can be put into the sample holder and be put in place. It is important to secure the slide onto the sample holder. In this case, we used poster stick gum, but a metal clasp would work even better. If the slides are not attached properly, they might move and cause drift in the image. This can also happen if immersion oil gets in between the slide and the sample holder. If there is drift, it is almost impossible to get the particles into focus and take a clear image. Therefore, care should be taken to avoid it.

After the slides are added to the microscope, about 30μl of the gold particle solution can be added to the flow cell. To allow the gold particles to settle, a certain amount of time should pass before taking measurements. I often chose a waiting time of about five minutes. After this time, enough particles had landed on the slide, and jiggling

particles had not stuck yet. The waiting time can be used to focus on the surface of the slide. The surface is recognisable, as there are often many particles visible on the same plane.

After the waiting time is up and the particles are in focus, the slide can be flushed with DI water, to remove the excess gold particles. The flushing is done by adding a small amount of DI water to one side of the flow cell, while simultaneously holding a piece of absorbent lens paper on the other side. Due to capillary forces, the gold particles are sucked out of the flow cell and the DI water is sucked in. After doing this three times, most of the excess gold will have been removed. Finally, the slides are ready for observation.

To visualise the particles and operate the microscope, several computer programs were needed. We mainly used HoKaWo imaging software to operate the camera. It can be used to view and record images, and to access camera settings. An important setting for our purpose is the image acquisition speed, or shutter time. This determines how long the camera takes to record one image. Usually a shorter shutter time is better, as it reduces image blurring caused by the movement of particles. The lower light intensity, which is a result of the shorter shutter time, can be compensated by increasing the laser power. To further sharpen the image, it is possible to pulse the laser associated with the microscope.

4.4.2 Laser pulsing

To improve image quality, the laser used in the microscope can be pulsed, to illuminate the sample for a very short time only. Without pulsing, the camera is the only thing which regulates the image acquisition. Effectively, the camera averages the input it gets during the acquisition of a single image. If the acquisition time is too long, the particles might have moved, causing the image to blur. To counteract this, the laser can be pulsed in sync with the camera, so the sample is only illuminated for a few nanoseconds. If the image is taken during the exact same time, it will be extremely clear and sharp. This is because there is nothing for the camera to average, there simply is only one shot of the illuminated slide.

To be able to do this pulsing, the setup needs to be altered slightly. We found that the easiest way to synchronise the laser and the camera is to use a wave generator which is attached to both. This ensures that the laser and camera turn on at the exact same time. Another advantage is that the wave generator settings can easily be changed during measurements, if anything needs to be adjusted.

Figure 6: Microscope picture of nanoparticles. The white specks on the screen are 40 nm gold particles. The illumination is best in the middle of the picture

5 Results

5.1 What to look for

An important part of this experiment is determining what a working nanospring will look like under the microscope. We expect that the particles will look like white specks on a black background, as the imaging is done with dark field microscopy. If particles are stuck to the glass, they will remain stationary. If they are connected to a nanospring, however, they will be moving due to Brownian motion. This means we will see them jiggling, as it is best described. The particles might move left to right slightly, and will also change their intensity. This intensity change is caused by the exponential decay of the evanescent wave. Looking at equation 8 shows that a change in the distance from the particle, z, results in a change in intensity. Thus, a jiggling particle will become randomly brighter and less bright over time. It turns out that this is quite hard to see by eye, so software has to be employed to determine this more definitively.

In figure 6, a standard microscope picture is shown. The white specks are the particles, in this case with a 40 nm diameter. Interesting to note is that the particles near the center of the image are brighter, because they are better illuminated. The total illuminated field is faintly visible too, this is a dark gray circle which just fits inside the picture. This circle indicates which area is illuminated by the laser. When setting up the microscope, this area is what you want to focus on.

5.2 Poly-l-lysine

To get a very rudimentary feeling of what particles under a microscope look like, we construct a test slide without nanosprings, but with poly-l-lysine on it. Poly-l-lysine, or PLL is an enzyme with a positive charge, which attaches to the glass of the slides. This is important, because most materials in water collect negative charge on their surface. The negative charges on the slide and the particles will repel each other, and they will not stick together. For this, PLL is used. After coating the glass slides by sandwiching them with the PLL solution in between, their charge becomes positive and they are attractive to the particles.

After coating the slides with PLL and making the flow cell, the diluted gold solution is added. Under the microscope, the results look similar to figure 6.

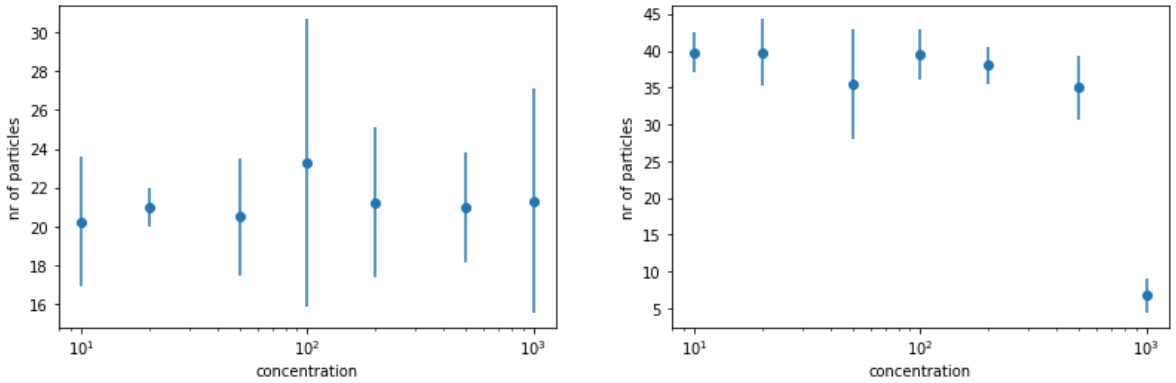
5.3 Ratio biotin

One of the first important properties to determine is the amount of PEG molecules to which a biotin head needs to be added. The amount of biotin-headed PEG compared to spacer PEG determines the amount of particles which can be seen through the microscope on a certain surface. Too little biotin, and the screen will be almost empty, too much and it will be crowded and the particles will be impossible to differentiate. We tried different concentrations, ranging from 1:100 to 1:100,000, to determine which one gave the preferred results.

In this case, a good result means a screen that has enough particles in it, without being too crowded. Ideally, the particles should also visibly jiggle, which indicates that they are attached to a PEG molecule, and not just stuck to the glass. Even if the particles' jiggling is hard to detect, the change in the amount of biotin can still teach us about specific and non-specific binding. If all the particles would bind to a nano-spring, adding more springs should result in more biotin. A steady increase of particles relative to the increase of biotin indicates proper binding, while a more random or less defined relation indicates that most particles did not bind to a molecule, but instead stuck to the glass.

5.4 PEG molecule length

One of the parameters which can be changed in this experiment is the length of the PEG molecules which are added to the slide. In the first round of experiments, both the biotin and non-biotin PEGs had a molecular weight of 5000. This corresponds with a length of about 32 nm. However, this did not work very well. There was a lot of non-specific binding, and there were no visible jiggling particles. I suspect this is because the molecules become tangled, and the biotin head might not be accessible to the gold particles. Next to this, the area where a particle would land is too crowded and full of filler PEG. Therefore, the particle cannot move properly, especially not when a potential will be added later.



(a) Filler PEG with a molecular weight of 5000

(b) Filler PEG with a molecular weight of 1000

Figure 7: Nr of counted particles, gold in 10 mM PB solution.

Therefore, a new type of filler PEG was used. Its molecular weight is 1000, which gives it a length of about 6.5 nm. Because of this shorter length, the filler PEG does not interfere with the attachment of the gold. The gold is also able to move freely above the space filled with spacer PEG. The results of these experiments are visible in figure 7. You can see that the amount of particles does not change significantly for the 5000 filler PEG, the amount of particles is relatively similar for every biotin ratio. I could not find any jiggling particles either. This indicates that there was no specific binding to the biotin.

The results for the short PEG are also not satisfactory. Even though the quantities seem somewhat more related, especially because of the low number of particles for the 1:1000 ratio, the amount of particles does not clearly increase with the amount of biotin added to the slide.

5.5 Gold solution

Another factor which greatly influences the amount of particles adhering to the sample slide, is the solution in which they are suspended. This solution will be a buffer with a certain salt concentration. This concentration determines how strong of an electric double layer is formed, and thus how strongly the particles and the glass slide attract each other. As explained above, particles dissolved in pure water will not attach to the plate at all, because both have a negative charge. When there are ions present though, these can form an EDL around the gold particles. Because of this, there is a layer of positive charge present, which makes the particles less repellent. They can come close enough to the slide to attach to a nanospring. If the ion concentration is too high though, the particles will be attracted to the slide too much, and they will stick to it very quickly. There will not be enough time for them to 'find' a nanospring to attach to. Thus, it is very important to get the balance exactly right.

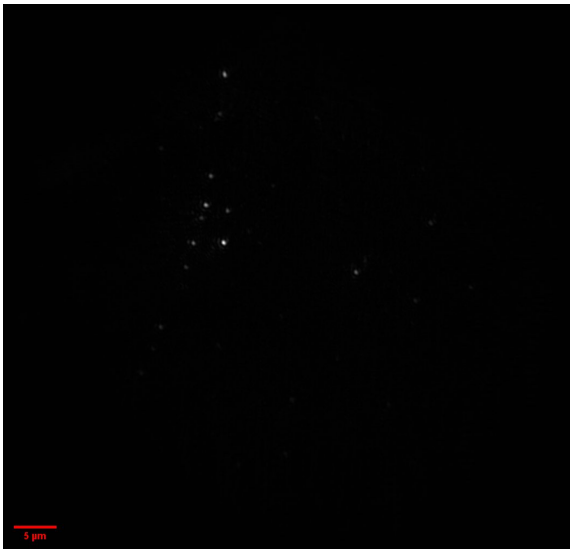
Next to this, the dilution of the particles is important as well. The particles come in a very highly concentrated solution, so they need to be diluted to work with them. In a low concentration, you will not waste as much gold, but there is a lower chance that a gold particle will attach to a spring, especially if the salt concentration is low as well. If there is a high concentration of gold however, there is a higher chance of attaching to a nanospring, though it might all stick to the plate if the salt concentration is too high.

The amount of particles sticking to the glass is determined by the salt concentration, which you therefore want to be as low as possible. The amount of particles sticking to the nanosprings will be positively influenced by the salt concentration as well though. To increase the chance of a particle binding to a spring without adding more salt, you can increase the concentration of gold particles in your solution. However, this increases the cost of the experiment quickly. Essentially, you want to have a gold concentration as high as you can afford, while simultaneously lowering the salt concentration such that there are not too many stuck to the glass, and enough to the springs. As you can imagine, this balance is quite hard to strike.

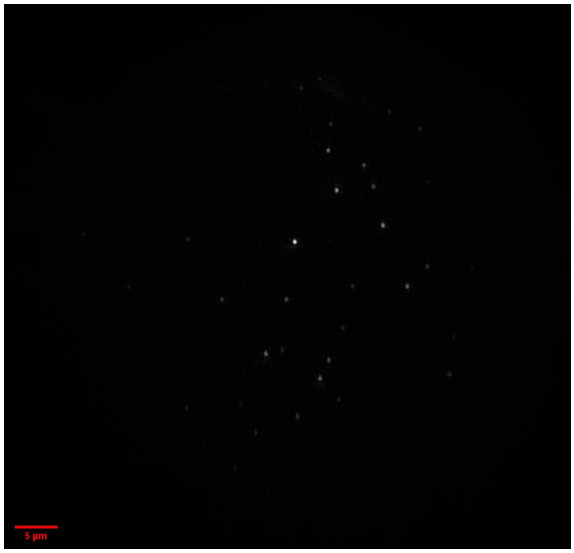
The gold particles originally come suspended in PBS. This stands for Phosphate-Buffered Saline, and typically has a molarity of 150 mM[18]. When diluting the particles 500x with PBS, we found that they all stuck to the surface extremely quickly. This meant that the salt concentration was too high. The amount of particles looked quite good though, so diluting them 500 times seemed okay.

To test which dilution would work better, we mixed several different molarities with two buffers present in the lab. There was a 10mM Phosphate buffer, and a 200mM phosphate buffer. By mixing these in different proportions, we created several solutions, of 20, 30, 50 and 70 mM. We then used each of those to dilute a small amount of the concentrated gold. Because the gold is originally diluted in PBS, this changed the molarity slightly, but not significantly. We tested all these solutions by adding them to 1:1000 biotin:non-biotin slides. When looking at them under the microscope, we could see the particles landing in real time. There was a big difference in the speed with which the particles landed. The lower molarities definitely took longer to land, so the effect of the salt concentration was clearly visible. After five minutes, we took pictures, which are visible in figure 8.

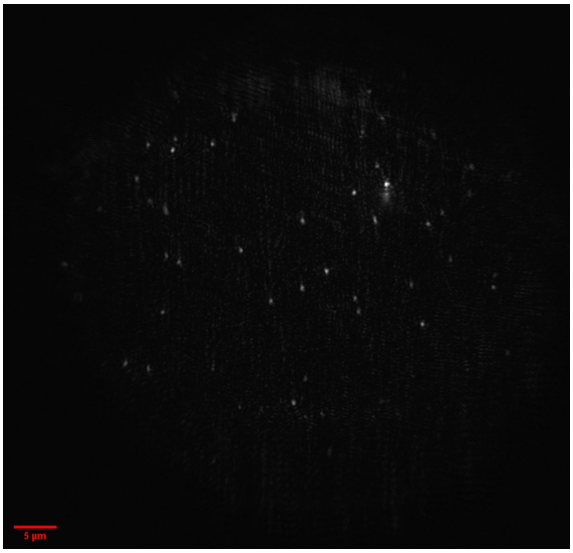
In figure 8a, where the 10 mM solution was used, there are fewer particles present than in the higher molarity solutions. The amount of particles in figure 8c and 8d are fairly similar, and landed very quickly during the experiment. Therefore we chose to not test the 70 mM solution, as this would only increase the unwanted effects. I say unwanted because there were no jigglers visible in any of the particle slides. Based on the theory above, however, it seemed most likely that a lower molarity would have a higher chance of producing jigglers, which is why we chose the 20mM solution to use for the further experiments.



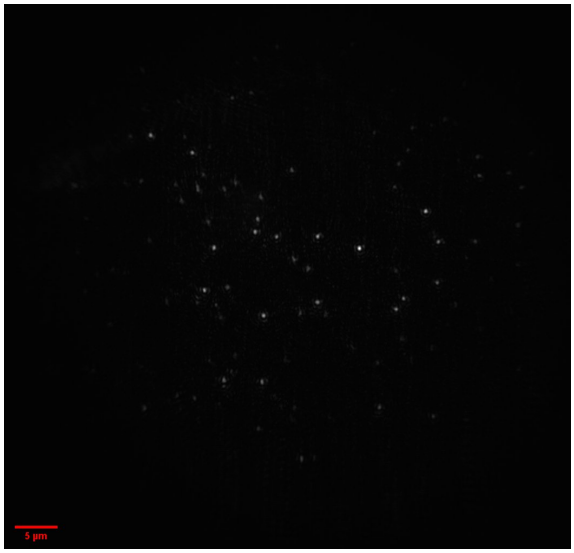
(a) 10 mM



(b) 20 mM

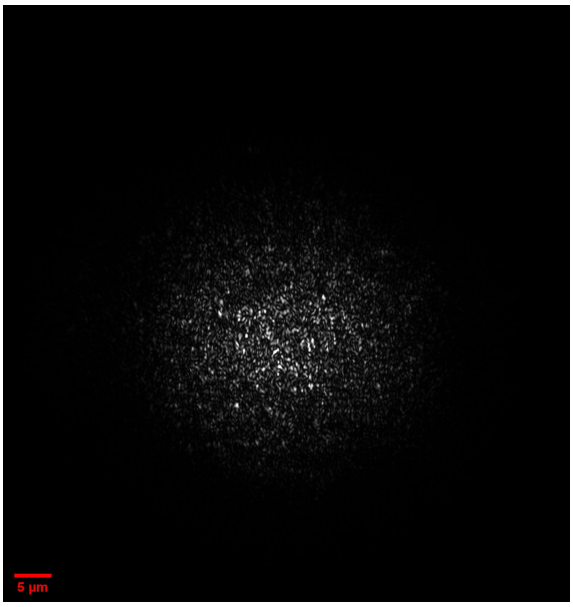


(c) 30 mM

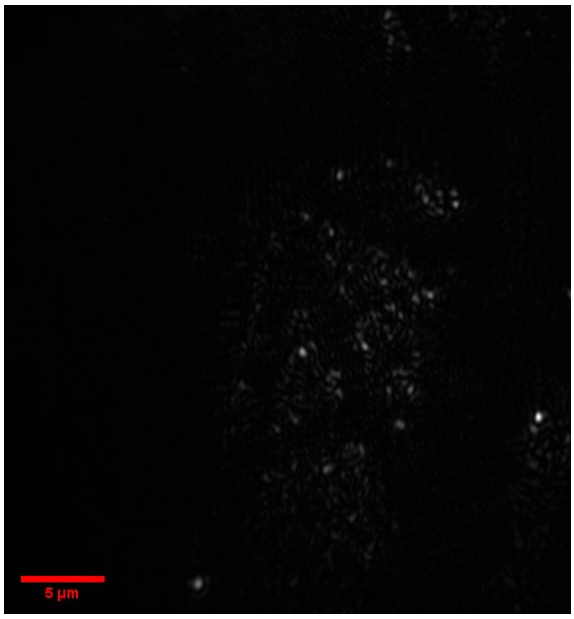


(d) 50 mM

Figure 8: Different molarities of gold solution on a 1:1000 plate, diluted 500 times



(a) A burnt part of the ITO surface



(b) An 'island' of excess ITO coating

Figure 9: Some pictures from the ITO slide, which show characteristic features.

5.6 ITO slides

The final goal of this experiment, after determining the different parameters described above, is to produce a sample of nanosprings on an ITO slide and apply a potential to it. We expect the particles to move up and down as the voltage changes, due to polarization[17]. This results in an intensity change, as explained in section 2.5.

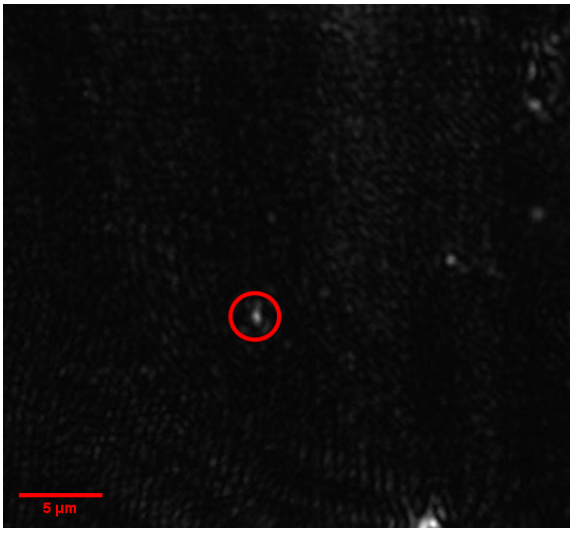
As described in section 5.6, coating an ITO slide in PEG molecules is similar to coating a glass slide. To apply a voltage to the slide, it is connected to a function generator. Ideally, the voltage can be monitored live, while looking at the microscope, but because of the limited time-frame this was not possible for us yet.

When looking at the ITO sample, some differences were noticeable immediately due to the nature of the ITO. In figure 9b, a characteristic 'island' of ITO is visible. This is caused by an excess of the crystal which forms while coating the slide with ITO. It is recognisable by its straight lines and angular edges, but it can be quite hard to differentiate from the gold particles which we are looking for. In figure 9a, a burnt part of the ITO surface is shown. It is characterised by a highly varied surface with different-sized speckles. This burn occurred because we accidentally shone a high powered laser on the surface for too long.

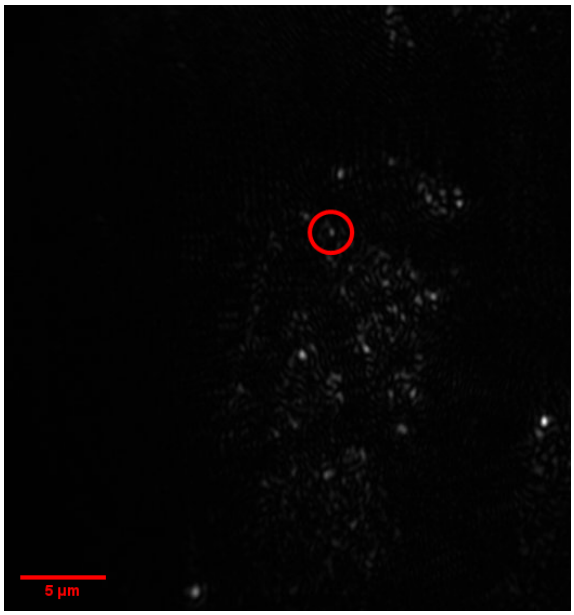
During this experiment round, several different voltages and frequencies were applied to the sample. We chose a relatively low frequency, of about 0.5 to 3 Hz, such that the movement of the particles could be visible by eye. If the frequency is very low, it might be overruled by brownian motion, but if it is too high it might not be discernible easily. Next to this, the voltage could be changed. We chose voltages around 1 V, as

this was enough to move the particles visibly, while not being too high. If the voltage was much higher, the particles would be attracted to the slide extremely strongly, and stick to the glass. They would not come loose anymore, rendering the slide useless for further observation.

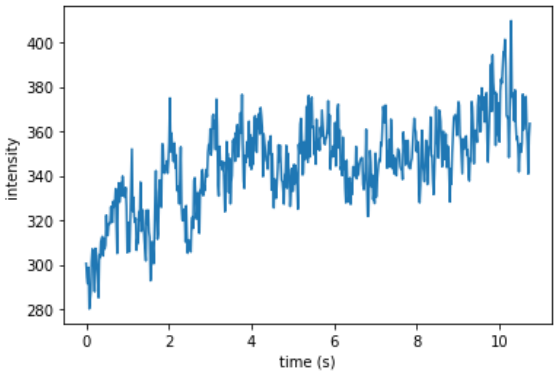
After looking at the slide under these different voltages, we discovered several jiggling particles which looked promising. To check if they were actually moving with the current, we performed some data analysis. The results are shown in figure 10. For the analysis, a particle was selected and its brightness was recorded for a certain period of time. The intensity of the light reflected by the particles is shown in figures 10c and 10d. In both cases, the particles had an electric field of 1 Hz applied to them. To see if this frequency was visible in the particles' movement, a fourier transform was performed. These results are shown in figures 10e and 10f. Unfortunately, there is no significant peak at 1 Hz, so it is not clear if these particles were actually influenced by the field as we had expected.



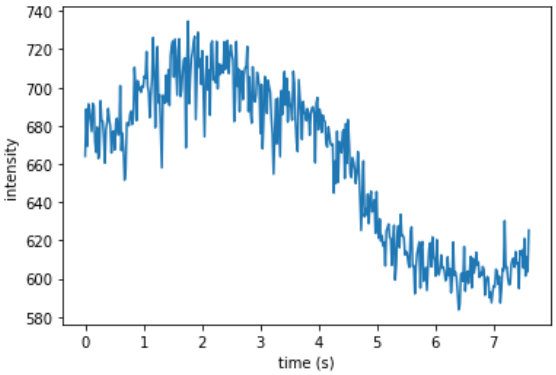
(a) Analyzed particle



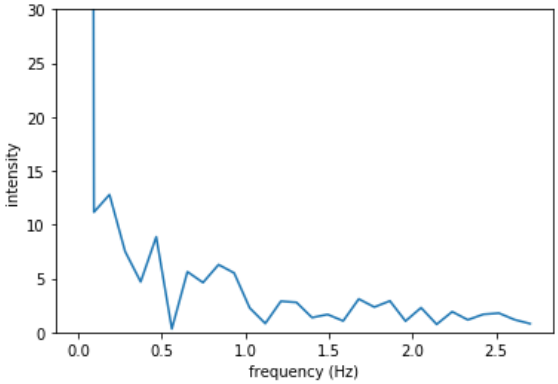
(b) Analyzed object



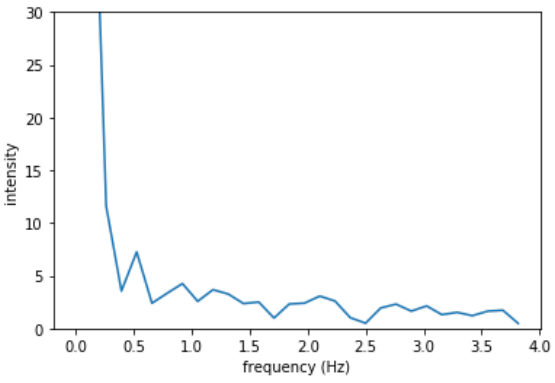
(c) Intensity plot over time, 1Hz, 2V



(d) Intensity plot over time, 1Hz, 2V

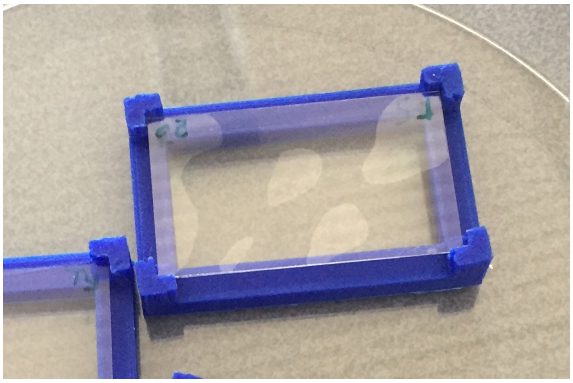


(e) Fast fourier transform, 1Hz, 2V



(f) Fast fourier transform, 1Hz, 2V

Figure 10: Analysis of particles on an ITO slide, with fourier transform



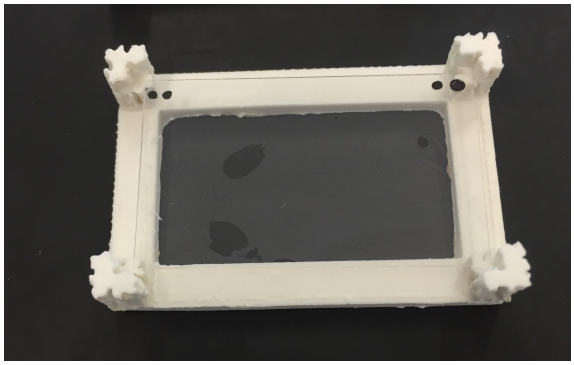


Figure 11: Two examples of slides which have not been coated properly.

6 Discussion

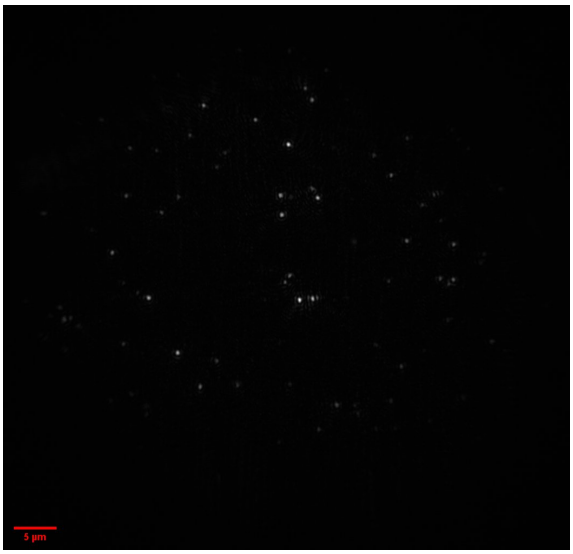
6.1 Slide construction

The quality of the PEG coating can vary slightly depending on how carefully the slides are constructed. If the slides are not sandwiched correctly, air bubbles might appear. If the slides are pressed together too hard, or too little liquid is added there might be areas of the slide which are not properly covered. When working too slowly, some of the liquid might already have evaporated, leaving a part of the slide without a coating. Finally, if the slides are taken out of the oven too early, they might not be completely dry, which might cause some spots where the PEG has not created a covalent bond with the glass.

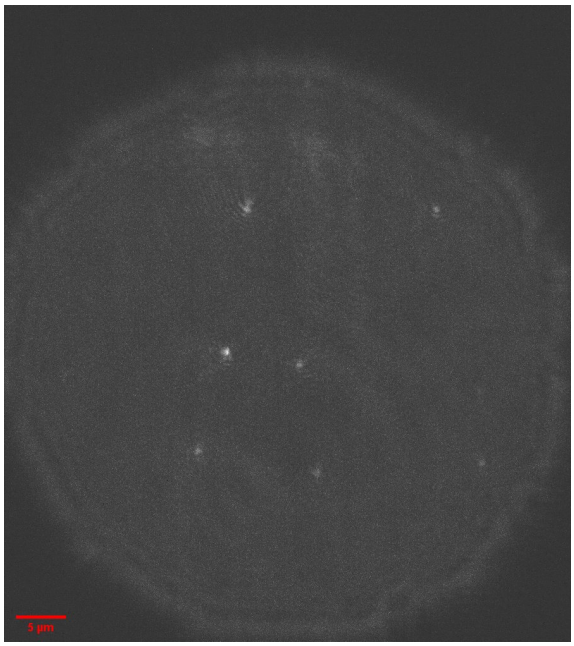
All these things can influence the quality of the final sample, and it is important to be careful to avoid them as much as possible. It might be worth it to check what 'good' and 'bad' parts of a finished slide look like, so mistakes can be recognised before the slide is used in the rest of the experiment. In figure 11 two examples of badly coated slides are shown. They show bubbles and lighter/darker patches and it is better to discard them.

6.2 Age of the slides

During the many rounds of experiments we conducted, we noticed that the time between the construction and the use of the slides was quite important for the result. Sometimes, we would coat the slides in PEG and use them later the same day, and sometimes there was a two day break in between. In figure 12 two pictures of a similar slide and salt concentration are shown. The difference between them is that we used one on the same day it was PEG-coated, and one two days after. It is clearly visible that the older slide has less particles attached to it. This might be because PEG is very sensitive to moisture, and leaving it out in the air, even when covered, might make the biotin head inoperable. To counteract this, the slides might be stored in a nitrogen cabinet or



(a) A 1:5000 slide used on the same day



(b) A 1:5000 slide from two days earlier

Figure 12: Slides with the same biotin ratio and salt concentration, used two days after producing them and on the same day

another stable environment, but the best solution is to use the slides as soon as possible after production.

6.3 ITO analysis

When analysing the ITO samples as described in section 5.6, we did not find the vibration frequency we had expected. The fourier transforms in figures 10e and 10f do not show the expected peak around 1 Hz. There might be several reasons for this. Firstly, it might be that the particles we analysed were of a different nature than we expected. Especially the particle in the right half of the figure might have been another type of object. This is because it might have been part of the excess ITO crystal which surrounds it. This is visible in figure 10b.

Another interesting result is the very clear wave which is visible in figure 10d. There seems to be a clear sinusoidal wave with a frequency of about 0.12 Hz, or a period of 8-9 s. It is unclear where this wave comes from. It might have to do with the way in which I analysed the data. To calculate the intensity, I added up the pixel value of the region where the particle was located. I divided this by the number of pixels to get an average value for each frame. This is what I plotted in the intensity graphs. However, there was a little bit of drift during the progression of the video. This means the particle moved out of the selected region if I chose it to have the exact size of the pixel. To avoid this, I took a region which was slightly larger than the particle, to contain it during the full

video. This lowered the total value of the intensity, but it might have influenced the results in another way as well.

6.4 Software

For this experiment, I used software that was quite basic and not extremely well-suited to my goals. For tracking and counting the particles, I used trackpy, a python package specifically designed for this purpose. There were several difficulties with using this package. Firstly, the particles were quite hard to select. Because the illumination of the field of view was not very homogeneous, parts of the photo were brighter than other. The contrast between the different videos changed quite a lot as well. This made it hard to set parameters within trackpy. The minmass, which is used to set the brightness features need to have to be counted, ranged from 5000 to 30000, and needed to be changed for almost every analyzed picture. This makes for very inaccurate data, so I ended up counting the particles by hand afterwards, as there usually were only a few present at the time.

I did not find any specific package for calculating the intensity of the particles over time. As explained in the previous paragraph, I had to manually select where the particles were located, and add up the pixel value of a small region to calculate the intensity. There are definitely improvements to be made here. The PyOCV software which is currently being developed at UU would be suitable for this. Ideally, this software would be able to operate the microscope, and scan across the sample until a jiggling particle is found. Up until now, this had to be done by hand, and it took quite some time. Next to this, the intensity of the particle should be measured accurately over time. Even if there is some drift, the particle should be followed so it can be analyzed for a longer amount of time. These features would make finding and analyzing particles easier and more accurate in the future.

7 Conclusion

As has been shown throughout this thesis, many factors play a role in creating a functioning nano-oscillator slide. After the experiments, the best results seem to come from a 1:1000 biotin ratio, combined with a 20 mM PB gold solution. After a waiting time of five minutes, some nano-oscillators might be observed, although they are scarce.

When applying a potential to the coated ITO slides, no clear results were found that the particles moved together with the electric field. In the future, this relation can be investigated further. To do this well, the software needs to be developed to include live intensity tracking, which makes quantifying data easier.

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Finally, I would like to thank my friends, with whom I have spent many hours inside writing during the past few months. Luckily, we had an exceptionally rainy spring this year, which I expect has helped me significantly.

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