Proof-of-concept of Thermally Contrasted Lateral Flow Assay Using Photothermal Heating From Magnetic Nanoparticles and Thermochromic Sheets for Limit of Detection Enhancement

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Chapter 1

Executive Summary

by Sean MacBride, Amrit Basi, Jennifer Lin

The aim of this project was to detect the lower limit of detection in nitrocellulose membranes when concentrated with various magnetic nanoparticles. Information on membranes, nanoparticles, thermochromic sheets, and applications for the research was gathered in the form of a literature review. Alongside background research, information about laboratory use and limitations were discussed with Prof. Nguyen and time slots were allocated with the appropriate risk assessments signed. This proved to be one of the most difficult tasks of the project, as UCL safety guidelines inhibited the use of the optimal laser. An appropriate laser on the second-floor lab within the UCL physics building with an excitation wavelength of 780nm and a power output of 80mW was used. The nitrocellulose membranes utilized were yellow and blue membranes from Bio-Rad with a pore size of 0.45μm and 0.2μm respectively.

Membranes with various concentrations of nanoparticle solution were synthesized. Initial tests were done with a membrane and thermochromic sheet setup without a thermal camera for empirical confirmation of the qualitative results which were observed as changes to the thermochromic sheet. Upon the conclusion of these tests, it was determined that the current setup would yield minimal qualitative results without the use of a thermal camera. The next round of tests was fifteen-minute exposures of the membranes at different concentrations. ~~Initial analysis of these results led us to believe that a three-minute exposure was sufficient to reach the maximum temperature of the membrane with a solution.~~ The third and final round of tests was with the three-minute exposure of membranes of various colors and solutions to the laser. This round of testing yielded the bulk of our results.

The data extracted from the tests was in the form of thermal images. The temperature of the hotspot was extracted using Testo IRSoft software. These temperatures were then analyzed and plotted using python and matplotlib [1].

Unfortunately, due to difficulty in initial hardware acquisition and limited test runs, data was inconclusive for both the detection of a lower limit of detection and a difference in heating between different membranes. Further test runs using these techniques would allow refinement of results with thermochromic sheets. The method used here could similarly be applied to lateral flow assays (LFA's), to allow for the testing of certain diseases and cultures in the body.

Furthermore, these results could be integrated into a mobile application where an easily accessible phone could be utilized to provide semi-qualitative results for LFA's, advising the user of appropriate action.

Chapter 2

Distribution of Work

Member	Work performed over course of project	Authorship pieces in report
Sean MacBride	Lab work and coordination, data analysis, and image testing	Executive summary, introduction, distribution of work, report formatting and editing
Grace Hymas	Lab work, data analysis, and poster work	Methods, discussion, report editing, and data analysis
Ivan Popov		
Amrit Basi		
Jennifer Lin	Literature review, report editing and formatting, diseases and infections presentation research, poster work	Executive Summary, Introduction, methods and materials, conclusion, final summary
Abigail Goh	Literature review, diseases and infections presentation research, email correspondence	Introduction, methods and materials
Anja Rellstab	Literature review, poster work, diseases and infections presentation research, app design	Introduction, conclusion
Wenhao Wu	Literature review, diseases and infections presentation research, email correspondence	Abstract, introduction, methods

Table 2.1: Table detailing the work done by individual group members on practical and logistical, laboratory, and report based work

Chapter 3

Introduction

by Abby Goh, Anja Rellstab, Wenhao Wu, Amrit Basi, Sean MacBride, and Jennifer Lin

3.1 Lateral Flow Assays

Point-of-care testing (POCT) allows for a prompt medical diagnosis at the time and place of a patient's care and as a result POCT has become one of the most popular methods of clinical analysis. LFAs are POCT based devices consisting of a sample pad, nitrocellulose membrane and test/control lines that can be used for detecting the presence or absence of pathogens, biomarkers or contaminants in a solution [2].

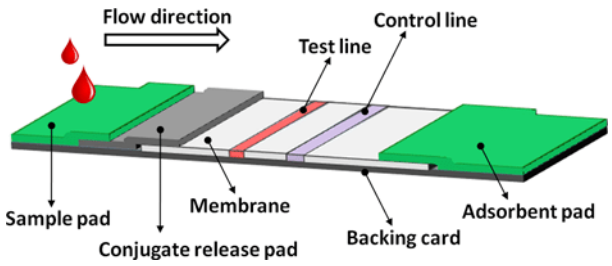

Figure 3.1: Illustration of a typical configuration of a Lateral Flow Assay composed of a sample pad, membrane, conjugate release pad, backing card, absorbent pad, test line and control line by Dr. Katarzyna M. Koczuła [2]

One of the most common forms of LFAs are pregnancy tests which test for the presence of anti-β hCG antibodies in urine. However LFAs have actually been employed in numerous different industries, as quality control tests and even used as a cheap alternative for early disease detection of several fatal diseases such as Dengue and Zika [3]. There have also been recent efforts to produce LFAs for multiplex detection LFAs, testing for 2 target analytes with one test, which could lead to testing of several diseases at a time.

LFAs are an ideal tool for detection due to their low development cost, prompt results, long shelf life and ease of use by patients without the help of a healthcare professional. Versatility is another component of LFAs as tests can be performed on various biological samples such as

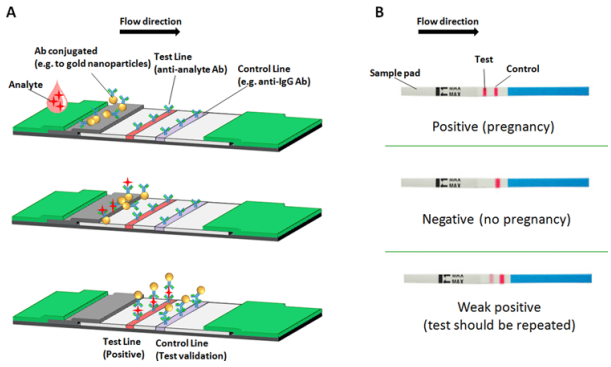

Figure 3.2: Illustration of the operation of a Lateral FLow Assay by (Dr.) Katarzyna M. Koczuła [2]

plasma, sweat, saliva, blood and more with only requiring a small sample for each test. These promising attributes mean LFA's are projected to increase in use at a Compound annual growth rate of 8.2% by 2022 [4].

Although LFAs have many advantages, there are several shortcomings such as inconsistent reproducibility, low biomolecule affinity, pre-treatment of the samples may be time consuming and the analysis time is dependent on the nature of the sample. One major disadvantage is its sensitivity and a number of possible solutions have been tested to address this such as the use of immunomagnetic particles and beads [5] [6], silver enhancement [7], magnetic nanoparticles and even SERS [9]. Many researchers are developing ways to increase sensitivity with microfluidics, biobar codes and enzyme based immunoassay technologies [8].

The aim of this project is to explore a new way of detection using nitrocellulose membranes in order to increase the sensitivity through the use of magnetic nanoparticles, excited by a laser, to quantify and increase the limit of detection of the target analyte. The limit of detection refers to the minimum concentration of the magnetic nanoparticles in which the result is distinguishable from the control results, i.e. a test strip absent of nanoparticles. This ultimately would lead on to a detection method that works as a lateral flow assay with greater sensitivity and where the change of colour in the thermochromic sheet reflects the concentration of analyte in the sample.

3.1.1 Thermally Contrasted Lateral Flow Assays

This experiment will be focusing on the Proof-of-Concept of thermally contrasted LFAs to improve sensitivity of LFAs. As this is the preliminary stages, no target antibody has been introduced yet and the solution used is purely magnetic nanoparticles of varying concentrations applied onto a nitrocellulose membrane. These coated membranes are then exposed to a laser, causing thermal activation in the ~~magnetic particles~~ and hence, an increase in temperature. In this experiment, a higher concentration of nanoparticles implies a higher concentration of antigen, hence it is expected that the temperature of the test strip increases with the concentration.

By inserting thermochromic sheets under the test strip membranes, the concentration of MNPs in the solution can be quantified. These thermochromic sheets shift colour due to the change in temperature and from the extent of temperature change they serve as a representation

of the concentration of the particles (and therefore also the analytes) visually. The colour change could be read both qualitatively and quantitatively by employing an optical strip reader or a software with a suitable colour calibration curve that is compatible on a mobile phone.

3.1.2 Types of Lateral Flow Assays

There are two main types of LFAs, sandwich assays and competitive assays. Sandwich Assays are used to measure the amount of analyte between two layers of antibodies. This is done by applying a known analyte to a plate and fixing it to the surface. Then, antibodies are introduced and bind with the analyte. The plate is washed and all unbound antibodies are removed. A second round of antibodies are introduced which now bind to the already formed analyte-antibody complexes. Once again all unbound antibodies are washed away and a substrate is added to produce either a fluorescent or chromogenic signal which can then be measured [9].

Competitive Assays are used to detect small analytes and work under the concept that unlabelled and labelled analytes compete with each other to bind with antibodies. Either the amount of unbound, labelled analyte is measured or this amount is washed off and only the bound analyte is measured [9]. The types of Lateral Flow Assays can be seen in figure 3.3 [10].

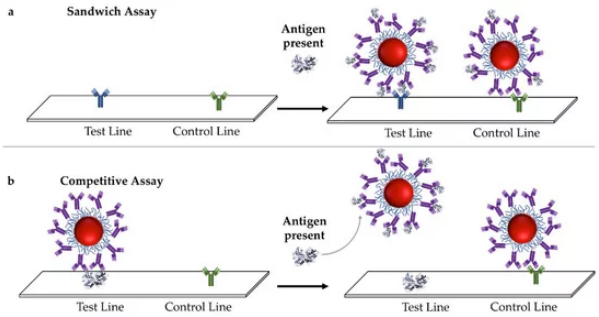

Figure 3.3: An illustration of a Sandwich assay and a Competitive assay with the following reaction in the presence of an antigen [10] .

LFAs for multiplexed detection are also being developed which could detect multiple target analytes simultaneously within one single test [11]. These Multiplex Assays work under the concept that colour coded magnetic beads, or microspheres, which are coated in specific antibodies and the analyte concentration can be distinguished by looking at the amount of each coloured beads at the end of the test [12]. They have yet to be tested commercially but look promising in the research scale [13]. This, along with further research could potentially remove or reduce the tendency for cross reactivity within LFA's.

3.2 Magnetic Nanoparticles

Nanoparticles are a diverse class of microscopic particles with units of dimensions measured in the nanometer scale. They are useful due to the fact that they bridge the gap between bulk materials and molecular structures due to their intermediate size. One main property of nanoparticles is their high surface area to volume ratio which provides high driving force in diffusion process,

especially at an elevated temperature. Due to the diffusive property,, the metallic nanoparticles bound with the analytes and achieve coalescence within a shorter time scale than bulk materials. This aggregation process is known as sintering where the small particles are welded together [14].

Nanoparticles are further classified by their size, shape, and material properties. The size of these particles typically ranges from 10nm 100nm which are classified as the chemical and physical properties are often size dependent [15]. The shape of the nanoparticles are also classified since it varies the surface area to volume ratio, therefore alters the behaviour in the sintering process. The shape classifications of nanoparticles is shown in 3.1. From the solid morphology classifications, 0D indicates particles with regular three dimensional shapes including spheres, 1D indicates nanorods, 2D indicates nanodisks, and 3D indicates nanostars. The synthesis of nanoparticles could be achieved by employing nucleation theory to control the growth rate of the particles. In addition, the shape of the nanoparticles could be engineered by altering the concentration of the growth agent which synthesises the nanoparticles [16]. With the variation of different sizes and selection of materials used, nanoparticles could be specifically engineered in application to various fields including cosmetics, renewable energies, and medicine [17].

Magnetic nanoparticles (MNPs) are a subclass of nanoparticles which have high magnetic susceptibility therefore interacts with the external magnetic field as they contain magnetic materials such as iron, cobalt, nickel, and their compounds. An example of magnetic nanoparticle is iron oxide. The magnetic property of MNPs also varies by particle size. At nanoscale dimension, iron oxide nanoparticles become superparamagnetic which is a useful property for biomedical usage as these particles align with the the magnetic field. Typical applications of MNPs include cancer treatments to target cancerous cells using magnetic resonance [18].

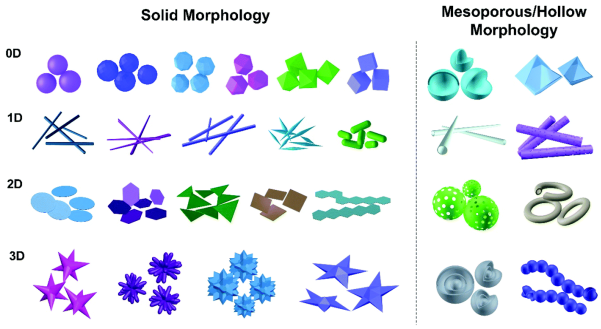

Figure 3.4: Nanoparticles shape classification [19].

3.3 Membrane Selection

Membranes are the focus of this experiment as they are one of the most essential parts of an LFA, in charge of allowing the sample to flow continuously through from the sample to absorbant pad and react with the antibody on the reaction area, thus shaping the initial sensitivity of the assay. Extensive research and preparation are needed before a membrane is selected and is ready for an experiment and there are several conditions taken into account when selecting the best membrane such as pore size, porosity and thickness. Pore size and porosity contribute to

determining the capillary flow rate.

As observed by H Shamloo Ahmadi et al [20], in current LFA designs that are studied, tested and available, the most commonly used membranes are the nitrocellulose membranes despite their fragility and high flammability. Although Polyvinylidene difluoride (PVDF) membranes seem to possess far superior properties, such as their higher binding capacity, sensitivity and durability. PVDF and nylon are not recommended for direct applications in LFAs [20] due to their natural properties including high hydrophobicity which needs activation before reacting with antibodies, and low capillary velocity, restricting the distance travelled by analyte.

Smaller pore sizes result in a slower capillary rate, but in turn provide a higher sensitivity by allowing for more time for target analytes to bind to the antibodies. Pore sizes smaller than 3μm however would be too slow to be effective in LFAs as discovered during membrane developments by Millipore [21]. Thickness and porosity control the amount of liquid required to fill the pore structure and ensure good signal visibility. Uniform wetting of the membranes is also essential for an accurate signal, to avoid significant discrepancies in output signal readings when measuring protein concentration in the target sample.

In a study by L.H Mujawar et al. [22], various brands of nitrocellulose membranes and their uniformity of biomolecule distribution were investigated to aid with membrane selection. Porous membranes, such as nitrocellulose, which is used in our experiment has a lower limit of detection as compared to 2D materials such as glass. By evaluating the printing and distribution of the antibody on membranes of 3 different manufacturers; GE Whatman, Sartorius-Stedim Biotech and Grace-Biolabs to analyse the uniformity of drops on each membrane. The distribution of the final signal was evaluated using the concentric ring overlay method by using high-speed cameras to investigate wettability and fluid flow and confocal laser scanning microscopy to evaluate the homogeneity of solution distribution both throughout the droplet and across the thickness of the membrane. Overall, Grace-Biolabs membranes produced the most homogeneous spots across the membrane which could be due to its hydrophobicity and fluid flow. However, due to time constraints, limited budget and a selective list of membrane suppliers, Bio-Rad membranes are used in this experiment.

With regards to membrane preparation, timing is crucial for membrane's humidity calibration. If the nitrocellulose membrane is too dry this results in non-uniform lines on the striping. If the membrane is too wet the test line is widened; consequentially the sensitivity and signal intensity are reduced as advised by Nanocomposix [23]. After equilibrating the membrane in a transfer buffer for 8-12 hours, the test and control lines can be imprinted onto the membrane, however, this striping is not required for the experiment in this project.

3.4 Quantifying Results with a Mobile Phone Application

Quantifying the LFA data and results is one of the hardest aspects of this experiment. Figuring out a way to do this so that it is easy, accessible and affordable would mean that there would be more future applications and could be introduced to health care in developing countries.

One company that has already developed an application compatible with Android and IOS

devices is Mobile Assay. Their application, a form of mobile diagnostic LFA test strip reader, allows users to read rapid diagnostic test results whilst also using cloud storage to collect their past data and get more detailed results online. The company currently targets their app towards smallholder farmers in Africa and their technology is specialised to test food safety by detecting toxic moulds in seeds and grains [24].

So far the contamination of Aflatoxin, Fumonisin, Filariasis, Onchocerciasis, Schistosomiasis as well as the endemic disease Malaria have been validated in this way. All of these maladies have severe adverse effects on humans and some can be fatal if left untreated.

Another smartphone lateral flow reader available is AppDx provided by Abingdon Health [25]. This application is also compatible with both Android and IOS devices and allows the user to set data criteria such as testing locations, times and dates. This criteria can also be used to help users pinpoint past data sets that have already been taken and stored by the application.

Both applications work under the same principle. The app is downloaded, a sample is provided for testing and the assay is run. The assay is then read by the phone or tablet and results are given within minutes and these can be synced between the cloud and other devices and stored.

To ensure accurate results, Mobile Assay has built in advanced light compensation and camera linearity to provide accurate results in different ambient conditions. They have also been able to pair their app with all rapid diagnostic tests, lateral flow test strips and also colour metric strips. Which proves that LFA's are very easily integrated into technology.

One very useful feature is that all tests are time stamped and geo-tagged allowing for farmers to pinpoint exact crops that have been tested on a map. Similar to AppDx, ranges can be set and notifications will alert a user to tests that come back outside of this allowed or safe range. Another great feature is the ability to export all date via DF, Excel or XML.

The backbone of being able to read the LFA's with digital technology comes from using Mobile Image Ratiometry (MIR) [26] and Quantitative Ratiometric Pixel Density Analysis (QRPDA). For MIR to produce accurate results a calibration or standard curve needs to be established so that all future results can be quantised by comparing the concentrations of analyte present in the current test with the calibration curves. To further analyse the concentrations more exactly the National Institute of Health has a processing software, Image-J [27], which allows users to subtract background, select specific signal bands, plot pixel density ratios of these bands and find the area under them to output the quantitative results [28].

3.5 Lasers and Nanoparticles in Tandem with Diagnostic Testing

Although LFAs have many advantages, there are several shortcomings such as inconsistent reproducibility, low biomolecule affinity, pretreatment of the samples may be time consuming and the analysis time is dependent on the nature of the sample. The major downfall is the sensitivity and several of the experiments above have addressed this in a number of ways, with the use of immunomagnetic particles and beads, silver enhancement, magnetic nanoparticles and even SERS. Many researchers are developing ways to increase sensitivity with microfluidics, biobar

codes and enzyme based immunoassay technologies [8]. In this experiment however, the sensitivity of LFAs hope to be improved by the use of magnetic nanoparticles, excited by a laser, to quantify and increase the detection limit of the target analyte.

To understand how magnetic nanoparticles could be employed with laser to improve detection limit, it is important to recall the mechanism of laser. Laser is a device which amplifies the original emitted electromagnetic radiation signal. The radiation emitted by a laser is monochromatic and carries momentum [29]. Optical radiation from the laser exerts radiation pressure as the momentum is transferred to an object. Monochromic signal prevents the dispersion effects caused by varied frequencies therefore ensures that the momentum transfer is consistent. The effect of the radiation pressure is amplified in small objects e.g. nanoparticles as these particles experience a larger acceleration due to the momentum transfer. Radiation pressure is a useful property of laser [30], and can be applied in such a way that allow optical tweezers to stretch and deform microscopic objects.

Of the nanoparticles mentioned above, magnetic nanoparticles interact directly with the electromagnetic radiation of the laser. As these particles are exposed to a laser, the nanoparticles oscillate such that they align with the external magnetic field [31]. These magnetic nanoparticles have a wavelength smaller than that of the radiation and so both the contribution from the oscillating magnetic field from the plane wave and the momentum transfer from the radiation to the particles results in the nanoparticles emitting thermal energy. This phenomenon is known as thermal activation where the temperature of the nanoparticle sample is increased [32]. This is important aspect in reading the LFA results as a higher concentration of nanoparticles implies a higher concentration of antigen, so the final temperature of the test strip will be higher than if the concentration was low.

3.6 Thermochromic Sheet Application

The sensitivity of the LFA's could be increased by inserting thermochromic sheets under the test strip membranes. These thermochromic sheets shift colour due to the change in temperature and from the extent of temperature change they serve as a representation of the concentration of the particles (and therefore also the analytes) visually. The colour change could be read both qualitatively and quantitatively by employing an optical strip reader or a software with a suitable colour calibration curve that is compatible on a mobile phone.

Chapter 4

Methods and Materials

by Grace Hymas, Abby Goh, Sean MacBride, and Wenhao Wu

4.1 Membrane Selection

Based on the manufacturers recommended by Nanocomposix and the type of products available on their websites, information was initially inquired on membranes made by MDI(Advanced Microdevices) from two different, pore size based application classifications listed by MDI. These membranes had pore sizes of 5 μm and 12 μm and flowing times of 180 sec and 150 sec. The membranes were also white in colour which was optimal as no laser absorption by the membrane would occur.

However due to the time limitations, 15 $\times$ 9.2 cm pre-cut nitrocellulose membranes of pore size 0.45 μm (yellow) and the 0.2 μm (blue) pore–size nitrocellulose membrane roll made by BIO-RAD were ordered. Since the hydrodynamical sizes of the nanoparticles used in experiment were around ~~100 nm~~, which is significantly smaller than our membrane pore size, the differences in magnitude between the initially selected membrane and the membrane that were actually ordered would not have an significant influence on the overall experimental results in terms of the point of interest in our investigation.

4.2 Membrane Preparation

In this experiment, Iron Oxide and Zinc Ferrite magnetic nanoparticles were used, with 2 different sizes for each type. The nanoparticle properties, mostly flow rate, will depend on size and so testing with different magnetic nanoparticles determined which ones were most favourable for this experiment. For Iron Oxide magnetic nanoparticles, small particles had a size of 10.40$\pm$2.60 nm while the large particles had a size of 13.2$\pm$2.10 nm. The Zinc Ferrite particles of large and small sizes were 15.13$\pm$2.25 nm and 11.00$\pm$1.80 nm respectively.

The magnetic nanoparticles were then diluted with water to produce 1000μl solutions of various different concentrations to find the detection limit in the following way. Using European Instruments Pipetman H3X018102 and CL50215 of volumes 0.5-100μl and 20-200μl respectively,

the required amount of water was extracted and deposited into Eppendorf tubes of 1.5ml. ~~The error on the pipette was stated to be 0.05μl.~~ A new pipette tip was used every time a new substance was introduced. The required amount of nanoparticles were deposited into the Eppendorf tube to complete the 1000μl of solution. ~~To ensure all the nanoparticles were deposited into the solution, the pipette was clicked while in the solution to expel any remaining nanoparticles in the pipette tip into the solution.~~ The Eppendorf tube was labelled with the magnetic nanoparticle size and concentration and placed into an SLS Lab Basics, 220-240V 44W Output 50-60Hz 0.4A vortexer for about 10 seconds to ensure homogeneity of the solution. This method was repeated for 20, 40, 50 and 80µg/ml concentrations for each Zinc Ferrite and Iron Oxide nanoparticles. A letter of the alphabet was assigned to each nanoparticle-concentration pair for ease of reference in labelling the membranes and storing data.

Once the solutions were produced, the membranes were prepared for nanoparticle loading. Membranes were cut into 2x3cm sheets, labelled with the correct letter of concentration and using a pipette, 50μl of solution was deposited onto each membrane sheet. The membranes were then left to dry, to ensure that no unnecessary particles remained and the particles were fixed onto the surface. In this experiment, membranes were dried in 2 different ways, overnight in a dry, dark environment and in a 55°C oven for 30 minutes. The preparation in which the membranes were heated in the oven for 30 minutes was implemented to reduce the time spent on membrane preparation. From the results, no tangible difference was observed between the 2 drying methods, only that more nanoparticles may have remained closer to the surface when dried in the oven and more may have sunk deeper into the membrane when dried overnight. This however did not affect the readings when the membranes were exposed to the laser.

50μl of solution of three types of nanoparticles known to absorb our particular laser wavelength were also deposited onto our blue and yellow membranes. These nanoparticles were 780nm Nanorods, 615nm Nanorods and 780nm Nanostars. These membranes were labelled separately to the alphabetised concentrations. Two control membranes were also prepared, one blue and one yellow membrane with no nanoparticles deposited.

There were some possible errors incurred in this method. Some solution was unavoidably left in pipette after transferring of each substance so the quantity of both nanoparticles and water was unlikely to be exactly correct. There was also likely human error on the volumes of solutions as the pipetting was done in stages and some stages could have been accidentally missed or miscounted. Parallax error on the pipette was another source of uncertainty, as well as air bubbles in the pipette and extra drops of nanoparticle or water on the outside of the pipette.

4.3 Experimental Setup

A class 3B 780nm laser with theoretical ~~input power 80mW~~ was mounted on a flat platform with mounting holes specialised for optical studies. The laser was set incident on a sample holder which was able to hold thin sheets - either thermochromic or membrane. The alignment ensured that the surface of the rectangular membrane piece held by the sample holder was perpendicular to the laser beam emitted, and the centre of the membrane piece where usually the nanoparticles

would be deposited was hit by the laser beam.

To analyse the change in temperature of the laser heated membranes, a Testo Ltd. 875-1i-InfraredCamera with SuperRespolution was used. The thermal imager was placed carefully in an angular position relative to the laser beam to avoid possible damages could be brought to the camera by the high intensity laser beam. The thermal imager was initially held by a human operator but was later mounted on a tripod at the edge of the platform to operate to avoid uncertainties introduced by hand movement. It was imperative that the distance between the membrane and laser was kept constant. A Tech Spin laser diode controller was used to control the intensity of a laser beam, as the output power of the laser initially fluctuated dramatically. This diode controller was essentially a semi-transparant mirror for attenuating the laser power. This was mounted along the line of propagation of the laser beam. An illustrative graph of this setup is as shown in Fig. 4.1 below.

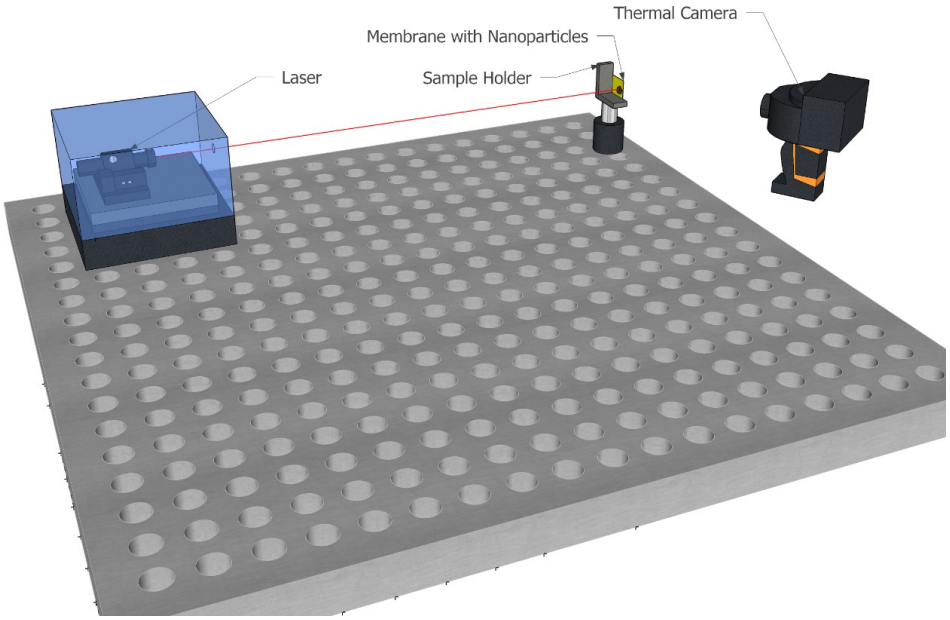

Figure 4.1: Experimental set up for measurement of increase in temperature of magnetic nanoparticles deposited onto a membrane when exposed to a laser.

4.4 Experimental Method: Membrane Laser Exposure

After membrane synthesis was completed, the membranes were mounted onto our sample holder as shown in Fig. 4.1. The output power of the laser was tested with a THOR LABS PM100D Optical Power and Energy Meter before every measurement and was shown to be averagely 31mW, with a fluctuation of around 5mW. This fluctuation was particularly prevalent at times close to when the laser was initially turned on, and seemed to take a small amount of time to 'warm up' and reach full power. After about one hour of so the fluctuations significantly

decreased, and after around 6 hours of measurement the laser began increasing higher than 31mW to around 33mW consistently. To combat this effect the laser was first turned on and left operating for 5 minutes before being used to heat the membrane. This allowed the laser functions to be stabilized and thus uncertainties due to laser intensity fluctuations over short run times could be reduced.

Preliminary tests were conducted with gold NanoRods with absorption wavelength 615nm and 780nm and NanoStars of absorption wavelength of 780nm. These wavelengths were within the frequency range of the laser and so these values were used as the control as a specimen for a strong positive result. In addition, NanoStars and NanoRods were selected since their larger surface area to volume ratio than the standard 0D spherical nanoparticles means they heat more efficiently. However this larger surface area causes the effect of aggregation is more profound [33].

Initial test runs to ensure that nanoparticles were correctly heating were performed for small Iron Oxide nanoparticles at 50μg/ml concentration on a blue membrane. This was compared to results of a control membrane. The initial temperature of each subject was 23.7°C. The nanoparticle membrane temperature increased from this ambient temperature to around 45°C after approximately 2 minutes of being exposed to the laser. The control membrane was exposed to the laser for 6 minutes and showed a temperature increase of only 0.4°C, thus our concept that a membrane with magnetic nanoparticles deposited onto it would heat more than one without was proved.

The following tests aimed to prove the limit of detection of each nanoparticle - the lowest concentration at which a temperature change of the membrane could be seen compared to one which contained no nanoparticles.

Thermochromic sheets were used to measure temperature change qualitatively for the next set of results. A 30 - 35°C temperature range thermochromic sheet was attached to the back of the chosen membrane with sticky tape. This membrane-sheet pair was mounted on the source holder such that the membrane was facing the laser. The laser was turned on such that its position could be seen, and it could be ensured that the laser was in fact incident on the nanoparticle region of the membrane as shown in Fig. 4.2 below. The output power of the laser was again recorded before every measurement. The laser was then turned on for 5 minutes, and the colour change of the thermochromic sheet was observed. If there was a very clear colour change after a short amount of time, the thermochromic sheet should have been changed to one with a higher range of activation, and if there was no change after a significant amount of time, the sheet altered to one with a lower range. This was repeated until no colour change was observed, and this range was assumed to be the excitation temperature of the nanoparticles. The results of this experiment can be seen in **??**.

The next experimental run was performed in the absence of thermochromic sheets. The same experimental method was used, with the run time set to 15 minutes and a thermal camera image taken every minute. These results showed a straight line trend with temperature readings being around the same for each time of recording, therefore it was inferred that a far shorter run time could be used for subsequent readings. As the temperature changes from these results were

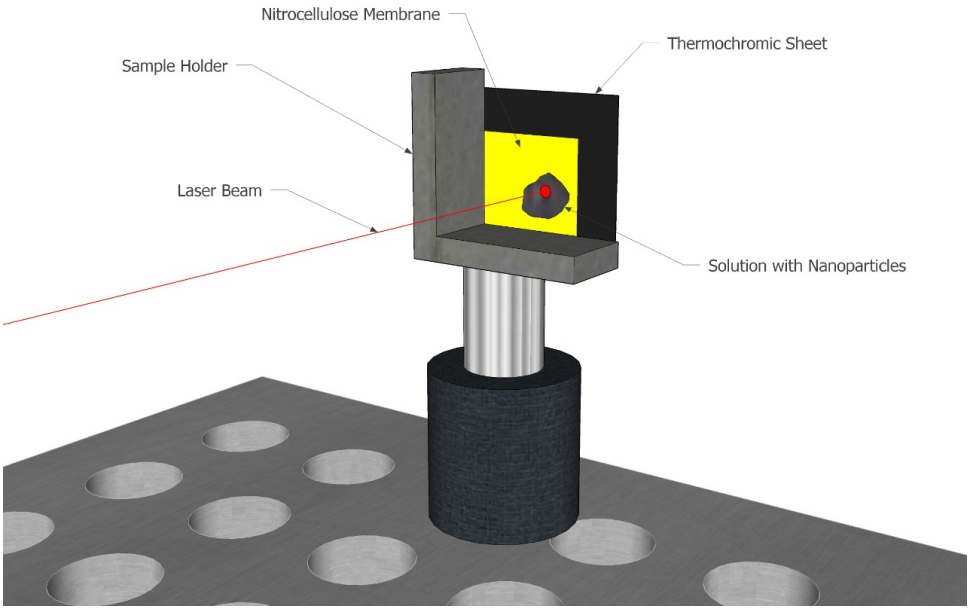

Figure 4.2: Alignment of mounted magnetic nanoparticles deposited onto a nitrocellulose membrane with a laser.

significant, it was decided that in the final session of results there should be 3 new concentrations of nanoparticles (5, 10 and 15 μg/ml) included which were lower than the ones tested before to investigate the limit of detection further. These results were recorded over 3 minutes for each membrane, with thermal images being taken every 30 seconds.

Chapter 5

Discussion

Chapter 6

Conclusion

conclusion here

Chapter 7

Acknowledgements

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Bibliography

[1] J. D. Hunter, "Matplotlib: A 2d graphics environment," *Computing In Science & Engineering*, vol. 9, no. 3, pp. 90–95, 2007.

[2] K. M. Koczula and A. Gallotta, "Lateral flow assays," *Essays In Biochemistry*, vol. 60, no. 1, pp. 111–120, 2016.

[3] M. Sánchez-Purrà, M. Carré-Camps, H. de Puig, I. Bosch, L. Gehrke, and K. Hamad-Schifferli, "Surface-enhanced raman spectroscopy-based sandwich immunoassays for multiplexed detection of zika and dengue viral biomarkers," *ACS Infectious Diseases*, vol. 3, no. 10, pp. 767–776, 2017.

[4] K. D. Jones, "Troubleshooting protein binding in nitrocellulose membranes, part 1: Principles," *IVD Technologies*, vol. 5, no. 2, pp. 32–41, 1999.

[5] Q. Li, H. Qi, Z. Zhong, H.-X. Zhou, C.-Y. Deng, H. Zhu, J.-F. Li, and X.-L. Wang, "A rapid and highly sensitive protocol for the detection of escherichia coli o157:h7 based on immunochromatography assay combined with the enrichment technique of immunomagnetic nanoparticles," *International Journal of Nanomedicine*, p. 3033, 2011.

[6] S. Workman, S. K. Wells, C.-P. Pau, S. M. Owen, X. F. Dong, R. LaBorde, and T. C. Granade, "Rapid detection of hiv-1 p24 antigen using magnetic immuno-chromatography (mict)," *Journal of Virological Methods*, vol. 160, no. 1-2, pp. 14–21, 2009.

[7] S. Rong-Hwa, T. Shiao-Shek, C. Der-Jiang, and H. Yao-Wen, "Gold nanoparticle-based lateral flow assay for detection of staphylococcal enterotoxin b," *Food Chemistry*, vol. 118, no. 2, pp. 462–466, 2010.

[8] Z. Qin, W. C. W. Chan, D. R. Boulware, T. Akkin, E. K. Butler, and J. C. Bischof, "Significantly improved analytical sensitivity of lateral flow immunoassays by using thermal contrast," *Angewandte Chemie International Edition*, vol. 51, no. 18, pp. 4358–4361, 2012.

[9] G. Biotech, "Sandwich elisa," 2019. [Online]. Available: https://www.genwaybio.com/services/sandwich-elisa

[10] D. R. Hristov, C. Rodriguez-Quijada, J. Gomez-Marquez, and K. Hamad-Schifferli, "Designing paper-based immunoassays for biomedical applications," *Sensors*, vol. 19, no. 3, 2019. [Online]. Available: http://www.mdpi.com/1424-8220/19/3/554

[11] M. ELSHAL and J. MCCOY, "Multiplex bead array assays: Performance evaluation and comparison of sensitivity to elisa," *Methods*, vol. 38, no. 4, pp. 317–323, 2006.

[12] S. X. Leng, J. E. McElhaney, J. D. Walston, D. Xie, N. S. Fedarko, and G. A. Kuchel, "Elisa and multiplex technologies for cytokine measurement in inflammation and aging research," *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, vol. 63, no. 8, pp. 879–884, 2008.

[13] K. Mohd Hanafiah, N. Arifin, Y. Bustami, R. Noordin, M. Garcia, and D. Anderson, "Development of multiplexed infectious disease lateral flow assays: Challenges and opportunities," *Diagnostics*, vol. 7, no. 3, p. 51, 2017.

[14] S. Magdassi, M. Grouchko, O. Berezin, and A. Kamyshny, "Triggering the sintering of silver nanoparticles at room temperature," *ACS Nano*, vol. 4, no. 4, pp. 1943–1948, 2010.

[15] G. Sergeev and K. Klabunde, "Size effects in nanochemistry," *Nanochemistry*, pp. 275–297, 2013.

[16] Z. Wu, S. Yang, and W. Wu, "Shape control of inorganic nanoparticles from solution," *Nanoscale*, vol. 8, no. 3, pp. 1237–1259, 2016.

[17] S. Gelperina, K. Kisich, M. D. Iseman, and L. Heifets, "The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 172, no. 12, pp. 1487–1490, 2005.

[18] A. Akbarzadeh, M. Samiei, and S. Davaran, "Magnetic nanoparticles: preparation, physical properties, and applications in biomedicine," *Nanoscale Research Letters*, vol. 7, no. 1, p. 144, 2012.

[19] Z. Wu, S. Yang, and W. Wu, "Shape control of inorganic nanoparticles from solution," *Nanoscale*, vol. 8, pp. 1237–1259, 2016. [Online]. Available: http://dx.doi.org/10.1039/C5NR07681A

[20] H. Shamloo Ahmadi, M. Heiat, H. Rashedi, and A. Latifi, 2015. [Online]. Available: http://www.biotechrep.ir/article_69167_21d318e6573b7563d30ad754cff7dd3f.pdf

[21] M. Corporation, "Rapid lateral flow test strips," 2002. [Online]. Available: https://www.emdmillipore.com/Web-US-Site/en_CA/-/USD/ShowDocument-Pronet?id=201306.12550

[22] L. H. Mujawar, A. A. Maan, M. K. I. Khan, W. Norde, and A. van Amerongen, "Distribution of biomolecules in porous nitrocellulose membrane pads using confocal laser scanning microscopy and high-speed cameras," *Analytical Chemistry*, vol. 85, no. 7, pp. 3723–3729, 2013.

[23] Nanocomposix, "Nitrocellulose membrane selection and striping for lateral flow assays," 2019. [Online]. Available: https://nanocomposix.com/pages/nitrocellulose-membrane-selection-and-striping-for-lateral-flow-assays

[24] "mobile diagnostic lateral flow test strip reader 2019," 2019. [Online]. Available: https://mobileassay.com

[25] A. Health, "Smartphone lateral flow reader oem," 2019. [Online]. Available: https://www.abingdonhealth.com/contract-services/smartphone-lateral-flow-reader-customisation/

[26] D. Cooper, D. Cooper, B. Callahan, P. Callahan, and L. Burnett, "Mobile image ratiometry: A new method for instantaneous analysis of rapid test strips," *Nature Precedings*, 2012.

[27] M. Abramoff, P. Magelhaes, and S. Ram, "Imagej," 2019. [Online]. Available: https://imagescience.org/meijering/publications/download/bio2004.pdf

[28] B. A. Cadle, K. C. Rasmus, J. A. Varela, L. S. Leverich, C. E. O'Neill, R. K. Bachtell, and D. C. Cooper, "Cellular phone-based image acquisition and quantitative ratiometric method for detecting cocaine and benzoylecgonine for biological and forensic applications," *Substance Abuse: Research and Treatment*, vol. 4, p. SART.S5025, 2010.

[29] H. Kogelnik and T. Li, "Laser beams and resonators," *Applied Optics*, vol. 5, no. 10, p. 1550, 1966.

[30] V. Letokhov and V. Minogin, "Laser radiation pressure on free atoms," *Physics Reports*, vol. 73, no. 1, pp. 1–65, 1981.

[31] M. Chu, Y. Shao, J. Peng, X. Dai, H. Li, Q. Wu, and D. Shi, "Near-infrared laser light mediated cancer therapy by photothermal effect of fe3o4 magnetic nanoparticles," *Biomaterials*, vol. 34, no. 16, pp. 4078–4088, 2013.

[32] H. Liao, C. L. Nehl, and J. H. Hafner, "Biomedical applications of plasmon resonant metal nanoparticles," *Nanomedicine*, vol. 1, no. 2, pp. 201–208, 2006.

[33] S. Nie and S. R. Emory, "Probing single molecules and single nanoparticles by surface-enhanced raman scattering," *Science*, vol. 275, no. 5303, pp. 1102–1106, 1997. [Online]. Available: http://science.sciencemag.org/content/275/5303/1102

Chapter 8

Appendix

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8.0.1 Python Scripts for Data extraction and Analysis

Fe_3O_4

```
    import pandas as pd
import matplotlib.pyplot as plt
import operator
import numpy as np
import scipy.optimize as optimization

from numpy import arange,array,ones
from scipy import stats

j=1
labels = np.array(["5\u03BCg/ml", "10\u03BCg/ml", "15\u03BCg/ml", "20\u03BCg/ml
    red↪ ", "40\u03BCg/ml", "50\u03BCg/ml", "80\u03BCg/ml"])
filename = "Control.csv" # the filename
df = pd.read_csv(filename) # breaking it up into appropriate lists
tmplist = df["Temp"].values.tolist()
yellowcontrol=tmplist[0:6]
bluecontrol=tmplist[6:]

filename = "BlueLarge.csv" # the filename
df = pd.read_csv(filename) # breaking it up into appropriate lists
bluelarge = df["Temperature"].values.tolist()

filename = "YellowLarge.csv" # the filename
df = pd.read_csv(filename) # breaking it up into appropriate lists
yellowlarge = df["Temperature"].values.tolist()
```

```
filename = "BlueSmall.csv" # the filename
df = pd.read_csv(filename) # breaking it up into appropriate lists
bluesmall = df["Temperature"].values.tolist()

filename = "YellowSmall.csv" # the filename
df = pd.read_csv(filename) # breaking it up into appropriate lists
yellowsmall = df["Temperature"].values.tolist()

timelist = [30,60,90,120,150,180]
for i in range(int(len(bluelarge)/6)):
    for j in range(6):
        bluelarge[6*i+j]=bluelarge[6*i+j]-bluecontrol[j]
for i in range(int(len(bluesmall)/6)):
    for j in range(6):
        bluesmall[6*i+j]=bluesmall[6*i+j]-bluecontrol[j]

for i in range(int(len(yellowlarge)/6)):
    for j in range(6):
        yellowlarge[6*i+j]=yellowlarge[6*i+j]-yellowcontrol[j]
for i in range(int(len(yellowsmall)/6)):
    for j in range(6):
        yellowsmall[6*i+j]=yellowsmall[6*i+j]-yellowcontrol[j]
plt.figure(figsize=(7,8))
for i in range(int(len(yellowsmall)/6)):
    plt.scatter(timelist,yellowsmall[6*i:6*(i+1)],label=labels[i])
    slope, intercept, r_value, p_value, std_err = stats.linregress(timelist,
        red↪ yellowsmall[6*i:6*(i+1)])
    point = [30*slope+intercept,180*slope+intercept]
    plt.plot([30,180], point)

plt.xlabel("Time (s)")
plt.ylabel("Differential Temperature ($^\circ$C)")
plt.grid()
plt.xlim(0,250)
plt.ylim(0,35)
plt.legend()

plt.title("Fe$_3$O$_4$ Small Nanoparticles on a Yellow Membrane")

plt.savefig("FeOsmallyellowTimevTemp.png",dpi=300)
```

```
plt.show()

plt.figure(figsize=(7,8))

for i in range(int(len(bluesmall)/6)):
    plt.scatter(timelist,bluesmall[6*i:6*(i+1)],label=labels[i])
    slope, intercept, r_value, p_value, std_err = stats.linregress(timelist,
        red↪ bluesmall[6*i:6*(i+1)])
    point = [30*slope+intercept,180*slope+intercept]
    plt.plot([30,180], point)

plt.grid()

plt.xlabel("Time (s)")
plt.ylabel("Differential Temperature ($^\circ$C)")

plt.xlim(0,250)
plt.ylim(0,15)

plt.legend()

plt.title("Fe$_3$O$_4$ Small Nanoparticles on a Blue Membrane")

plt.savefig("FeOsmallblueTimevTemp.png",dpi=300)

plt.show()

plt.figure(figsize=(7,8))

for i in range(int(len(yellowlarge)/6)):
    plt.scatter(timelist,yellowlarge[6*i:6*(i+1)],label=labels[i])
    slope, intercept, r_value, p_value, std_err = stats.linregress(timelist,
        red↪ yellowlarge[6*i:6*(i+1)])
    point = [30*slope+intercept,180*slope+intercept]
    plt.plot([30,180], point)

plt.grid()

plt.xlabel("Time (s)")
plt.ylabel("Differential Temperature ($^\circ$C)")
plt.legend()
```

```
plt.xlim(0,250)
plt.ylim(0,35)

plt.title("Fe$_3$O$_4$ Large Nanoparticles on a Yellow Membrane")

plt.savefig("FeOlargeyellowTimevTemp.png",dpi=300)

plt.show()

plt.figure(figsize=(7,8))

for i in range(int(len(bluelarge)/6)):
    plt.scatter(timelist,bluelarge[6*i:6*(i+1)],label=labels[i])
    slope, intercept, r_value, p_value, std_err = stats.linregress(timelist,
        red↪ bluelarge[6*i:6*(i+1)])
    point = [30*slope+intercept,180*slope+intercept]
    plt.plot([30,180], point)

plt.grid()

plt.xlabel("Time (s)")
plt.ylabel("Differential Temperature ($^\circ$C)")

plt.xlim(0,250)
plt.ylim(0,20)

plt.legend()

plt.title("Fe$_3$O$_4$ Large Nanoparticles on a Blue Membrane")

plt.savefig("FeOlargeblueTimevTemp.png",dpi=300)

plt.show()

plt.figure(figsize=(7,8))

for i in range(int(len(bluelarge)/6)):
    plt.scatter(timelist,bluelarge[6*i:6*(i+1)],label=labels[i])
    slope, intercept, r_value, p_value, std_err = stats.linregress(timelist,
        red↪ bluelarge[6*i:6*(i+1)])
    point = [30*slope+intercept,180*slope+intercept]
```

```
    plt.plot([30,180], point)

plt.grid()

plt.xlabel("Time (s)")
plt.ylabel("Differential Temperature ($^\circ$C)")

plt.xlim(0,250)
plt.ylim(0,20)

plt.legend()

plt.title("Fe$_3$O$_4$ Large Nanoparticles on a Blue Membrane")

plt.savefig("FeOlargeblueTimevTemp.png",dpi=300)

plt.show()

plt.figure(figsize=(7,8))

cap=3
s=5
l=0.7

concs=[5,10,15,20,40,50,80]

# small nanoparticles on yellow

smallyellowavgs=[]
smallyellowstd=[]
for i in range(int(len(yellowsmall)/6)):
    avg=np.mean(yellowsmall[6*i:6*(i+1)])
    std=np.std(yellowsmall[6*i:6*(i+1)])
    smallyellowavgs.append(avg)
    smallyellowstd.append(std)
slopesy, interceptsy, r_value, p_value, std_err = stats.linregress(concs,
    red↪ smallyellowavgs)
point = [5*slopesy+interceptsy,80*slopesy+interceptsy]
plt.plot([5,80], point,color="r",linestyle="--")
plt.scatter(concs,smallyellowavgs,label="Small Fe$_3$O$_4$ on Yellow",color="r",
    red↪ s=s)
```

```
plt.errorbar(concs,smallyellowavgs,smallyellowstd,linestyle="none",color="r",
    red↪ elinewidth=l,capsize=cap)

# small nanoparticles on blue

smallblueavgs=[]
smallbluestd=[]
for i in range(int(len(bluesmall)/6)):
    avg=np.mean(bluesmall[6*i:6*(i+1)])
    std=np.std(bluesmall[6*i:6*(i+1)])
    smallblueavgs.append(avg)
    smallbluestd.append(std)
slopesb, interceptsb, r_value, p_value, std_err = stats.linregress(concs[0:3],
    red↪ smallblueavgs)
point = [5*slopesb+interceptsb,15*slopesb+interceptsb]
plt.plot([5,15], point,color="b",linestyle="--")
plt.scatter(concs[0:3],smallblueavgs,label="Small Fe$_3$O$_4$ on Blue",color="b
    red↪ ",s=s)
plt.errorbar(concs[0:3],smallblueavgs,smallbluestd,linestyle="none",color="b",
    red↪ elinewidth=l,capsize=cap)

# large nanoparticles on yellow

largeyellowavgs=[]
largeyellowstd=[]
for i in range(int(len(yellowlarge)/6)):
    avg=np.mean(yellowlarge[6*i:6*(i+1)])
    std=np.std(yellowlarge[6*i:6*(i+1)])
    largeyellowavgs.append(avg)
    largeyellowstd.append(std)
slopely, interceptly, r_value, p_value, std_err = stats.linregress(concs,
    red↪ largeyellowavgs)
point = [5*slopely+interceptly,80*slopely+interceptly]
plt.plot([5,80], point,color="m",linestyle="--")
plt.scatter(concs,largeyellowavgs,label="Large Fe$_3$O$_4$ on Yellow",color="
    red↪ gold",s=s)
plt.errorbar(concs,largeyellowavgs,largeyellowstd,color="m",linestyle="none",
    red↪ elinewidth=l,capsize=cap)

# large nanoparticles on blue
```

```
largeblueavgs=[]
largebluestd=[]
for i in range(int(len(bluelarge)/6)):
    avg=np.mean(bluelarge[6*i:6*(i+1)])
    std = np.std(bluelarge[6*i:6*(i+1)])
    largeblueavgs.append(avg)
    largebluestd.append(std)
slopelb, interceptlb, r_value, p_value, std_err = stats.linregress(concs[0:3],
    red↪ largeblueavgs)
point = [5*slopelb+interceptlb,15*slopelb+interceptlb]
plt.plot([5,15], point,color="c",linestyle="--")
plt.scatter(concs[0:3],largeblueavgs,label="Large Fe$_3$O$_4$ on Blue",color="c
    red↪ ",s=s)
plt.errorbar(concs[0:3],largeblueavgs,largebluestd,color="c",linestyle="none",
    red↪ elinewidth=l,capsize=cap)

plt.grid()
plt.legend()
plt.xlabel("Concentration (\u03BCg/ml)")
plt.ylabel("Mean Temperature ($^\circ$C)")
plt.title("Concentration vs. Mean Temperature for Fe$_3$O$_4$ on different
    red↪ membranes")

plt.savefig("FeOConcentrationvMeanTemp.png",dpi=300)

plt.show()

plt.figure(figsize=(7,8))

labels = ["5\u03BCg/ml large","5\u03BCg/ml small","10\u03BCg/ml large","10\
    red↪ u03BCg/ml small","15\u03BCg/ml large","15\u03BCg/ml small"]

for i in range(int(len(bluelarge)/6)):
    diff=np.array(bluelarge[6*i:6*(i+1)])-np.array(yellowlarge[6*i:6*(i+1)])
# slope, intercept, r_value, p_value, std_err = stats.linregress(timelist,
    red↪ bluelarge[6*i:6*(i+1)])
# point = [30*slope+intercept,180*slope+intercept]
# plt.plot([30,180], point)
    plt.plot(timelist,diff,label=labels[2*i])

    diff=np.array(bluesmall[6*i:6*(i+1)])-np.array(yellowsmall[6*i:6*(i+1)])
```

```
    plt.plot(timelist,diff,label=labels[2*i+1])
plt.xlabel("Time (s)")
plt.ylabel("T$_B$-T$_Y$ ($^\circ$C)")
plt.title("Temperature difference between Blue and Yellow membranes with
    red↪ Fe$_3$O$_4$")
plt.grid()
plt.legend()

plt.savefig("TempDiffFeO.png",dpi=300)

plt.show()

# large yellow
largeyellow=-interceptly/slopely
# small yellow
smallyellow=-interceptsy/slopesy
# large blue
largeblue=-interceptlb/slopelb
# small blue
smallblue=-interceptsb/slopesb
print(largeyellow,smallyellow,largeblue,smallblue)
```

$\mathbf{Zn}_0.4\mathbf{Fe}_2.6\mathbf{O}_4$

```
    import numpy as np
import matplotlib.pyplot as plt
%matplotlib inline
# this comand will create graphs in Jupyter notebook # rather than in a separate
    red↪  window
concentration, data_y1_orig = np.loadtxt("ZnFeO_small_yellow.csv", delimiter
    red↪ =';', unpac k=True) # upload data from a file
concentration, data_y2_orig = np.loadtxt("ZnFeO_large_yellow.csv", delimiter
    red↪ =';', unpac k=True) # upload data from a file
concentration, data_y3_orig = np.loadtxt("ZnFeO_small_blue.csv", delimiter=';',
    red↪ unpack= True) # upload data from a file
concentration, data_y4_orig = np.loadtxt("ZnFeO_large_blue.csv", delimiter=';',
    red↪ unpack= True) # upload data from a file
data_x1 = np.array([5, 10, 15, 20, 40, 50, 80]) # create an array of
    red↪ concentration val ues
data_x2 = np.array([5, 15, 20, 40, 50, 80]) # create an array of concentration
    red↪ value s
```

```
data_x3 = np.array([10, 15, 20, 40, 50, 80]) # create an array of concentration
    red↪ values data_x4 = np.array([5, 10, 15, 20, 40, 80]) # create an array
    red↪ of concentration values
control1 = np.array([24, 23.3, 23.8, 23.5, 23.6, 23.4]) # create an array of
    red↪ yellow co ntrol temp values
control2 = np.array([24.3, 24.7, 25.3, 25, 24.6, 24.8]) # create an array of
    red↪ blue cont rol temp values
col = np.array(['b', 'r', 'g', 'm']) # array with colour codes for plotting
    red↪ shape = np.array(['o', 's', '^', '*'])
legends = np.array(["$Zn_{0.4}Fe_{2.6}O_4$ small, yellow membrane",
"$Zn_{0.4}Fe_{2.6}O_4$ large, yellow membrane", "$Zn_{0.4}Fe_{2.6}O_4$ small,
    red↪ blue membrane", "$Zn_{0.4}Fe_{2.6}O_4$ large, blue membrane"])
# subtract the control values from the actual temperature values
j=0
data_y1 = np.zeros(len(data_y1_orig))
# every 6 values in data_x array start subtracting from the first value # of the
    red↪  control array again
for i in range(0, len(data_y1_orig)):
data_y1[i] = data_y1_orig[i] - control1[j] j += 1
if(j == 6):
j=0
j=0
data_y2 = np.zeros(len(data_y2_orig))
# every 6 values in data_x array start subtracting from the first value # of the
    red↪  control array again
for i in range(0, len(data_y2_orig)):
data_y2[i] = data_y2_orig[i] - control1[j] j += 1
if(j == 6):
j=0
j=0
data_y3 = np.zeros(len(data_y3_orig))
# every 6 values in data_x array start subtracting from the first value # of the
    red↪  control array again
for i in range(0, len(data_y3_orig)):
data_y3[i] = data_y3_orig[i] - control2[j] j += 1
if(j == 6):
j=0
j=0
data_y4 = np.zeros(len(data_y4_orig))
# every 6 values in data_x array start subtracting from the first value # of the
    red↪  control array again
```

```
for i in range(0, len(data_y4_orig)):
data_y4[i] = data_y4_orig[i] - control2[j] j += 1
if(j == 6):
j=0
# find the average temperature and error for each concentration
data_y1_ave = np.zeros(7)
data_y2_ave = data_y3_ave = data_y4_ave = np.zeros(6)
data_y1_ave_err = np.zeros(7)
data_y2_ave_err = data_y3_ave_err = data_y4_ave_err = np.zeros(6)
for i in range(0, 7):
data_y1_ave[i] = np.mean(data_y1[(i*6):((i+1)*6)]) data_y1_ave_err[i] = np.std(
    red↪ data_y1[(i*6):((i+1)*6)])
for i in range(0, 6):
data_y2_ave[i] = np.mean(data_y2[(i*6):((i+1)*6)]) data_y2_ave_err[i] = np.std(
    red↪ data_y2[(i*6):((i+1)*6)])
for i in range(0, 6):
data_y3_ave[i] = np.mean(data_y3[(i*6):((i+1)*6)]) data_y3_ave_err[i] = np.std(
    red↪ data_y3[(i*6):((i+1)*6)])
for i in range(0, 6):
data_y4_ave[i] = np.mean(data_y4[(i*6):((i+1)*6)]) data_y4_ave_err[i] = np.std(
    red↪ data_y4[(i*6):((i+1)*6)])
# define a function that will be plotting least square fit straight lines
def least_square_fit(data_x, data_y, data_y_err, j):
mean_x = np.mean(data_x) # calculate mean value of x coordinates mean_y = np.
    red↪ mean(data_y) # calculate mean value of y coordinates
m = np.sum((data_y - mean_y)*data_x) / np.sum((data_x - mean_x)*data_x) c =
    red↪ mean_y - m*mean_x
n = len(data_x) # calculate the number of pairs of coordinates D_calc = np.sum((
    red↪ data_x - mean_x)**2)
sum_d2 = np.sum((data_y - m*data_x - c)**2)
dm = np.sqrt(sum_d2 / (D_calc*(n-2))) # calculate uncertanity in m
    dc = np.sqrt(((1/n) + (mean_x**2/D_calc)) * (sum_d2 / (n-2)))
anity in c
    # create two arrays
    fit_x = np.linspace(np.min(data_x), np.max(data_x), 2)
    fit_y = m * fit_x + c
    # plot the line
    plt.plot(fit_x, fit_y, '--', color=col[j])
    # plot the data points
# calculate uncert
```

```
    plt.errorbar(data_x, data_y, fmt=shape[j], yerr=data_y_err, color=col[j],
        red↪ label=leg
ends[j])
plt.figure(figsize=(7,8))
least_square_fit(data_x1, data_y1_ave, data_y1_ave_err, 0)
least_square_fit(data_x2, data_y2_ave, data_y2_ave_err, 1)
least_square_fit(data_x3, data_y3_ave, data_y3_ave_err, 2)
least_square_fit(data_x4, data_y4_ave, data_y4_ave_err, 3)
#plt.errorbar(data_x4, data_y4_ave, fmt='o', yerr=data_y4_ave_err, color=col[3],
    red↪  label= legends[3])
# set the graph parametres
plt.grid(True)
plt.title("Concentration vs. Mean Temperature for $Zn_{0.4}Fe_{2.6}O_4$ on
    red↪ different me mbranes")
plt.legend(loc="lower right")
plt.xlim(0, 100)
plt.ylabel("Mean Temperature, \u00b0C")
plt.xlabel("Concentration, \u03BCg/ml")
plt.savefig("ZnFeO_conc_vs_temp.png", dpi = 300)
data_y4 = np.append(data_y4[:6], data_y4[12:])
data_y2 = np.append(data_y2[:24], data_y2[30:])
time = np.array([30, 60, 90, 120, 150, 180])
new_data_y1 = data_y3[:] - data_y1[6:]
col = np.array(['b', 'r', 'g', 'm', 'c', 'k']) # array with colour codes for
    red↪ plotting labels = np.array(["10\u03BCg/ml", "15\u03BCg/ml",
"20\u03BCg/ml", "40\u03BCg/ml", "50\u03BCg/ml", "80\u03BCg/ml"])
# define a function that will be plotting least square fit straight lines
def least_square_fit1(data_x, data_y, j):
mean_x = np.mean(data_x) # calculate mean value of x coordinates mean_y = np.
    red↪ mean(data_y) # calculate mean value of y coordinates
m = np.sum((data_y - mean_y)*data_x) / np.sum((data_x - mean_x)*data_x) c =
    red↪ mean_y - m*mean_x
n = len(data_x) # calculate the number of pairs of coordinates D_calc = np.sum((
    red↪ data_x - mean_x)**2)
sum_d2 = np.sum((data_y - m*data_x - c)**2)
dm = np.sqrt(sum_d2 / (D_calc*(n-2))) # calculate uncertanity in m
    dc = np.sqrt(((1/n) + (mean_x**2/D_calc)) * (sum_d2 / (n-2)))
    # create two arrays
    fit_x = np.linspace(np.min(data_x), np.max(data_x), 2)
    fit_y = m * fit_x + c
    # plot the line
```

```
    plt.plot(fit_x, fit_y, '-', color=col[j])
    # plot the data points
    plt.plot(data_x, plot_y, 'o', color=col[j], label = labels[j])
# calculate uncertanity in c
plt.figure(figsize=(7,8))
# plot data points 7 times
start = 0
end = 6
for i in range(0, 6):
plot_y = new_data_y1[start:end] least_square_fit1(time, plot_y, i) start = end
end += 6
# set the graph parametres
plt.grid(True)
plt.title("Temperature Difference Between Blue and Yellow mwmbranes with $Zn_
    red↪ {0.4}Fe_ {2.6}O_4$")
plt.legend(loc="lower right")
plt.xlim(0, 250)
plt.ylabel("$T_B-T_Y$, (\u00b0C)")
plt.xlabel("Time (s)")
plt.savefig("Temp_diff_small.png", dpi = 300)

import numpy as np
import matplotlib.pyplot as plt
%matplotlib inline
# this comand will create graphs in Jupyter notebook # rather than in a separate
    red↪  window
concentration, data_y = np.loadtxt("ZnFeO_large_yellow.csv", delimiter=';',
    red↪ unpack=True ) # upload data from a file
control = np.array([24.3, 24.7, 25.3, 25, 24.6, 24.8]) # create an array of blue
    red↪  contr ol temp values
data_x = np.arange(30, 181, 30) # create an array of time values
#data_x = np.tile(data_x, 7)
col = np.array(['b', 'r', 'g', 'm', 'c', 'k']) # array with colour codes for
    red↪ plotting labels = np.array(["5\u03BCg/ml", "10\u03BCg/ml", "15\u03BCg/
    red↪ ml",
"20\u03BCg/ml", "40\u03BCg/ml", "80\u03BCg/ml"])
# subtract the control values from the actual temperature values
j=0
data_y_corr = np.zeros(len(data_y))
# every 6 values in data_x array start subtracting from the first value # of the
    red↪  control array again
```

```
for i in range(0, len(data_y)):
data_y_corr[i] = data_y[i] - control[j] j += 1
if(j == 6):
j=0
# define a function that will be plotting least square fit straight lines
def least_square_fit(data_x, data_y, j):
mean_x = np.mean(data_x) # calculate mean value of x coordinates mean_y = np.
    red↪ mean(data_y) # calculate mean value of y coordinates
m = np.sum((data_y - mean_y)*data_x) / np.sum((data_x - mean_x)*data_x) c =
    red↪ mean_y - m*mean_x
n = len(data_x) # calculate the number of pairs of coordinates D_calc = np.sum((
    red↪ data_x - mean_x)**2)
sum_d2 = np.sum((data_y - m*data_x - c)**2)
dm = np.sqrt(sum_d2 / (D_calc*(n-2))) # calculate uncertanity in m
    dc = np.sqrt(((1/n) + (mean_x**2/D_calc)) * (sum_d2 / (n-2)))
anity in c
    # create two arrays
    fit_x = np.linspace(np.min(data_x), np.max(data_x), 2)
    fit_y = m * fit_x + c
    # plot the line
    plt.plot(fit_x, fit_y, '-', color=col[j-1])
    # plot the data points
# calculate uncert
plt.plot(data_x, plot_y, 'o', color=col[j-1], label = labels[j-1])
plt.figure(figsize=(7,8))
# plot data points 7 times
start = 0 end = 6 j=1
for i in range(0, 6):
plot_y = data_y_corr[start:end] least_square_fit(data_x, plot_y, j) start = end
end += 6
j += 1
# set the graph parameters
plt.grid(True)
plt.title("$Zn_{0.4}Fe_{2.6}O_4$ Large Nanoparticles on a Blue Membrane") plt.
    red↪ legend(loc="upper right")
plt.xlim(0, 250)
plt.ylabel("Differential Temperature, \u00b0C")
plt.xlabel("Time, s")
plt.savefig("ZnFeO_large_blue.png", dpi = 300)
```

8.0.2 Finances

8.0.3 Minutes