Detecting Nanoparticle Motion In Tissue via Magnetic Spectroscopy

Aleksander Gornik[1 2], Theo Desbrousses[1 6], Roy Kongnyuy[1 3], Morwenna Maunder[1 2], Frederick Watkins[1 4], David Bolarinwa[1 5] and Ollie Harper[1 2]

[1] *Cambridge Consultants,United Kingdom*
[2] *Loughborough University,United Kingdom*
[3] *Imperial College London,United Kingdom*
[4] *Cambridge University,United Kingdom*
[5] *Warwick University,United Kingdom*
[6] *Bath University, United Kingdom*

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Abstract— Magnetic spectroscopy of Brownian motion (MSB) is a technique that allows scientists to detect concentrations of molecular biomarkers. MSB uses the harmonics of the magnetization induced by a low frequency oscillating magnetic field to provide quantitative information about magnetic nanoparticles. In addition to this, nanoparticles can be functionalized – modified with molecules to enable specific interactions with target substances. In this case a biomarker is used which changes the rotational freedom of the nanoparticle. This means that detection of a bound nanoparticle equates to the detection of the biomarker. The rotation of the nanoparticle is significant. When a nanoparticle is bound it will have reduced rotational freedom which induces a greater change in magnetisation response. This is what was studied during this project. This research focuses on human tissue, specifically in developing technology capable of in-body sensing of certain biomarkers however due to its innovative nature more information about the application for this cannot be disclosed due to confidentiality issues. The harmonics in this research and more specifically, the ratio between the 3rd and 5th harmonics of a superparamagnetic iron oxide nanoparticle (SPION) not only provide evidence that a biomarker is detected but also provide information about how a specific nanoparticle-biomarker pair is rotating. These results show clear proof of concept that it is possible to create a non-invasive method of sensing biomarkers in human tissue.

Keywords— Nanoparticles, biomarkers, functionalized, harmonic.

I. Introduction

The The popularity of SPION's has steadily grown since the 1990's when researchers started investigating their use in biological systems. Since the boom of the biotech sector these nanoparticles have been used in magnetic resonance imaging (MRI), magnetic particle imaging (MPI) and other optical methods to functionalize SPION's with specific dyes for their detection optical signals. This project uses SPION's for biosensing purposes. The plan is to develop a handheld medical device that is safe for patient use and can detect low concentrations of nanoparticle and biomarker solution through human tissue. The biomarker is a proxy for a biological process of interest. The objective of this project was to develop a coil that can produce a magnetic field strength of 10mT which in turn can decrease the freedom of rotational motion of the nanoparticle. (Xiaojuan Zhang a, 4 July 2013) A value of 10mT was chosen for two reasons . The first is simplicity – It is known that this value is proven to work from research cited above. Secondly – It is known that most iron oxide nanoparticles need around 10-20mT of field strength to ensure saturation. SPION's have a good track record in recent literature for biosensing applications in conjunction with streptavidin and biotin pairs which are known to be biological binding pairs. The challenge was making a coil that can provide a fairly uniform field and is safe to use. Many designs were considered, initially a Helmholtz coil to maximise uniformity however a simpler wound coil prototype was made as the team discovered that the uniformity does not need to be as stable as we initially anticipated. Creating a coil that can create sufficient field strength and complies to health and safety regulations was a difficult task and therefore an accurate simulation model was necessary to accelerate software development and give a better understanding of what an accurate nanoparticle signal looks like. In addition, this simulation model was used for testing purposes as a ref-

Contact data: Aleksander Gornik, aleksandergornik@gmail.com

erence to how accurately the coil system was constructed. As part of the project a live demonstration of the technology was required. To do this, a program was developed which exploits the use of audible frequencies. The rig produced a constant frequency that the audience can hear and once the ratio between the two desired harmonics changes – the frequency changes. The change of frequency will be representative of amount of change detected: a high concentration of nanoparticle will be more distinct to the human ear compared with an in-vitro concentration. Furthermore, a basic and intuitive UI was built using TKinter in python for calibration and measurement purposes of a multi-purpose instrument tool that worked as a waveform generator and spectrum analyser.

II. Methods

a. Biology

The primary biology challenge was selection of the correct proxy biomarker. The technology that the team built allowed for flexibility in the choice of biomarker which means that this device will be a multi-purpose biosensing device. Proxy biomarkers are measurable indicators of a biological state or condition, so can be used to indicate whether the device is working. The proxy chosen was streptavidin due to its highly reliable and well understood binding nature with biotin, which acts like an antibody to detect the biomarker.

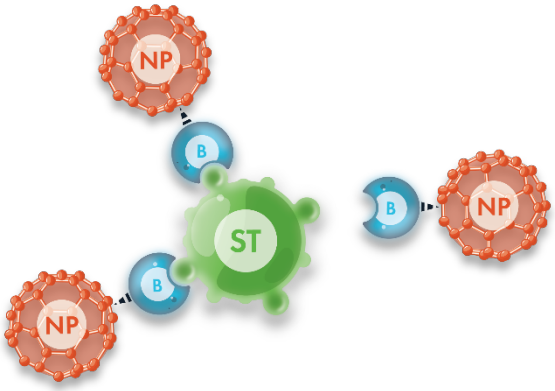

Fig. 1: Streptavidin bound to two biotin's

Streptavidin is composed of four subunits and so has four biotin binding sites. This structure therefore allows several nanoparticles to bind to the same molecules o that they form chains (or polymers). The outcome is reduced rotational freedom of the nanoparticles which magnifies the magnetisation response. In addition, research was conducted into which hydrogels provide a representative in-body environment for the nanoparticles. Suitable hydrogels must have a pore size large enough to enable free movement of nanoparticles, while having a fabrication method compatible with in-house manufacture. Agarose and alginate had pore sizes that were far too small. Polyethylene glycol (PEG) and collagen required complex fabrication methods. The most suitable hydrogel found was fibrin, which is made from mixing fibrinogen (a soluble protein found in blood plasma) and thrombin (an enzyme in blood plasma). Nanoparticles were added to the hydrogels by mixing them with the fibrinogen solution first and then adding the thrombin.All biological protocols mentioned above are from the research paper (Xiaojuan Zhang a, 4 July 2013).

A robust protocol was developed to conduct nine experiments (including one control). Nine petri dishes were sourced. In each one a base concentration of PBS (phosphate buffered saline – a buffer) and polysorbate-20 (to extend the response time for the signal). Each petri dish contained a different concentration of streptavidin. As the concentration of streptavidin increased, an increase in the ratio of the relevant harmonics was expected. In addition to these experiments, tests with hydrogels were also planned. This involved a control that only contained hydrogel and another with nanoparticles and hydrogel.

b. Hardware

1. Coil Theory

By designing a coil which can apply the correct magnetic field strength onto the nanoparticle, an accurate, filtered, and non-distorted output signal will be generated. Graeser et al. have described several methods to achieve this. The method of choice was the cancellation method. The most accurate method, the combined method, was capable of achieving a high attenuation for suppressing the excitation signal and still recover the full particle signal including the base frequency. However, as we were interested in only harmonic 3 and 5, this degree of accuracy was not necessary.

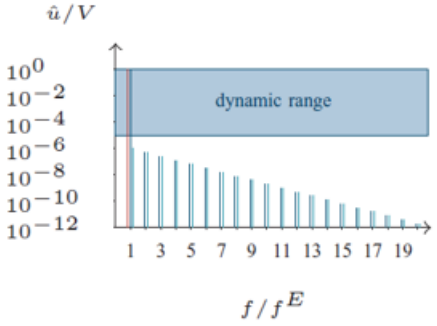

Fig. 2: Dynamic range without filtering

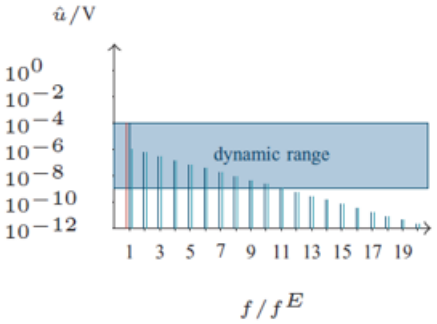

Fig. 3: Dynamic range with cancellation coil

Figure 2[1] shows the achievable dynamic range without filtering of the desired spectrum. Figure 3 is a revised version using the cancellation method that allows us to extract up to around the 9th harmonic with a good degree of accuracy. Based on this information we chose this approach.

[1]Theory and images are all from (Matthias Graeser, 20 February 2013).

In our case, we have iron oxide nanoparticles which needs to be exited by a single excitation field. This field oscillates in a purely sinusoidal manner and therefore has a frequency f_E. Due to inductive coupling, the excitation signal induces a voltage signal $u^E(t)$ in the receive coil which is a pure sine wave. Due to nonlinear magnetization behaviour, the nanoparticle signal $u^p(t)$ induced in the receive coil experiences a non-sinusoidal distortion. Because of this, the frequency spectrum of $u^p(t)$ is rich in harmonics that decays exponentially. Now applying the principle of superposition, we can theorise that the total signal in the receive coil is:

$$u(t) = u^E(t) + u^p(t) \tag{1}$$

From this formula we understand that we need to suppress $u^E(t)$. To do this the cancellation method can be used and so the inverse of $u^E(t)$ needs to be detected. A gradiometer receive coil can achieve this. This cancellation coil is identical to the sensing coil but has an opposite winding direction. By connecting the two coils together in series we can identify another parameter that can be defined as $u^C(t) \approx -u^E(t)$. This is turn creates a new formula expressed as:

$$u(t) = u^E(t) + u^p(t) + u^C(t) \tag{2}$$

The key in cancelling out this distortion, to be left with $u(t) = u^p(t)$, is to create a coil with sufficient accuracy. In this case accuracy is the degree to which the result will be representative of the correct value. The correct value will be masked under a noise and distortion which needs to be eliminated.

2. Coil Design

The cancellation method was applied and figure 4 depicts the high-level sensor design, which is split into two separate coils: the receive (Rx) coil and transmit (Tx) coil. The input signal is sent to the transmit coil that produces a magnetic field which excites the nanoparticles and the receive coil senses the nanoparticle behaviour as the output signal. Rx has two windings connected in series. The clockwise sensor winding (group B) detects a signal $u(t)$ where $u(t) = u^E(t) + u^P(t)$ and the anticlockwise cancellation winding (group A) produces $u^C(t) \approx -u^E(t)$. As they are in series the theory of superposition takes effect.

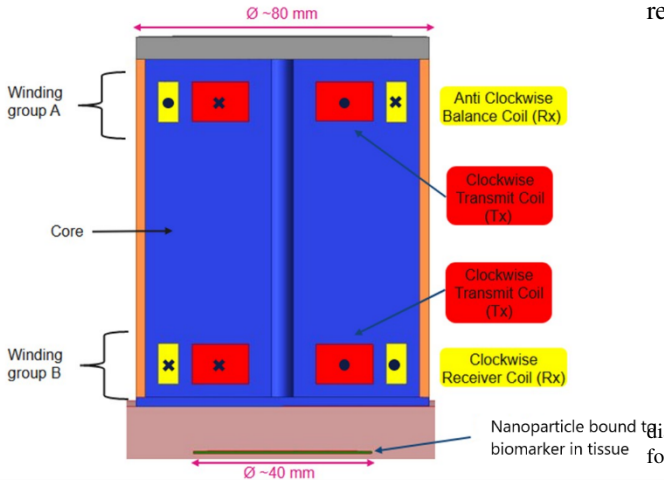

Fig. 4: Coil Design

When placed in a time varying magnetic field, SPION's have a maximum signal output determined by their [2]intrinsic properties. Therefore, to maximise signal strength, the sensor's magnetic field strength should saturate the SPION's. This field strength was determined to be 10mT. To excite the SPION's, a clockwise transmit winding capable of generating an alternating 10mT peak amplitude field strength with a 1300 drive frequency was required. The transmit winding excites the SPION's which vibrate and generate their own magnetic field. The SPION's alternating magnetic field induces a measurable current in the sensing coil (group B). A challenge here is that the transmit coil also couples with the sensing coil, thereby inducing an additional current in the receiver coil. This additional current drowns out the SPION-induced current and increases the dynamic range. To overcome this and essentially reduce the dynamic range, a second set of windings are required to act as an analogue filter before the software and electronics. The current induced in the sensor winding (group B) by the Tx coil is equal in magnitude, equal in time period, and 180° out of phase with the current induced in the receive winding by the Tx coil. Therefore, when superimposing the output signal from the group A and B coils, the current induced by Tx is removed. Leaving only the current induced by the SPION's.

Requirements List:

1. Magnetic Field strength through nanoparticles: Sinusoidal, 10mT peak field strength, 1300Hz frequency.
2. Identical coupling (LCR values) between Rx and Tx windings.
3. [3]Output signal in the low tens of mV.
4. Feasible to hand wind with access to coil terminals.
5. Must be safe to conduct experiments as an operator.

A preliminary design used a 21A peak sinusoidal current, operating at 1600W RMS, this design approach provided clear manufacturing advantages (less turns in the coils and so less chance for inaccuracies, producing tighter LCR tolerances). However, the coil was later redesigned at a lower current (4.24 A RMS) and voltage (42.4 V RMS) for two reasons:

1. Health and Safety. The coil would have to operate at currents exceeding 30mA, as such the operating voltage must be below 50V rms to prevent electrocution. The device casework should be insulating (ABS) to prevent physical contact with the coils during operation.
2. Power. Due to amplifier and signal generator inefficiencies, drawing 1600 W from a mains socket was too high and would necessitate the use of a class B amplifiers, increasing signal to noise ratio.

[2]These properties consist of μ, the nanoparticle magnetic moment, the diameter of the SPION and other properties like density which can all be found on the datasheet of the specific nanoparticles

[3]The way this estimate was found is shown in the nanoparticle modelling section.

c. Software

1. Modelling Nanoparticle Behaviour

Building the coil was an essential part of this project and an output signal was necessary to understand how the software team will approach signal processing. To go around this issue, the team simulated how nanoparticles aggregate in MSB detection of biomarkers. (Dhrubo Jyoti 1, 4 September 2022). The researchers in the paper cited were able to calculate an experimentally measurable magnetic signal using the model:

$$\frac{dM}{dt} = -\frac{2M}{\tau_b}\left[1 - \frac{\zeta_0 \sin(\omega t)}{\alpha_e(t)}\right] \tag{3}$$

Where τ_b is a constant which represents Brownian relaxation time as:

$$\tau_b = \frac{3\eta V}{k_b T} \tag{4}$$

which depends on viscosity η and hydrodynamic volume V and:

$$\zeta_0 = \frac{\mu H_0}{K_B T} \tag{5}$$

Equation (3) is ultimately what needs to be solved. The solution to this will provide a magnetic signal as a function of time. ζ_0 is a constant which consist of μ, the nanoparticle magnetic moment, H_0 which is the applied field amplitude, K_B being the Boltzmann constant and T being the temperature in kelvin. Now, the [4]Langevin function can be introduced to define the effective field $\alpha(t)$ in terms of M so the ODE is less intimidating. The Langevin function is:

$$L(\alpha) = \coth(\alpha) - \frac{1}{\alpha} = M(t) \tag{6}$$

Using Pade's rounded approximation (Jedynak, 2015) we can define the inverse of the Langevin as:

$$\alpha(t) = L^{-1} \approx M\frac{3 - m^2}{1 - m^2} \tag{7}$$

And therefore:

$$\frac{dM}{dt} = \frac{2M}{\tau_b}\left[1 - \frac{\zeta_0 \sin(\omega t)}{M\frac{3-M^2}{1-m^2}}\right] \tag{8}$$

Which simplifies to:

$$\frac{dM}{dt} = \frac{1}{\tau_b}\left[2M - \frac{2(1-m^2)}{3-m^2}\zeta_0 \sin(\omega t)\right] \tag{9}$$

This simplified model is now solvable computationally using iterative methods. This assisted coil simulations and provided insight as to what was expected which allowed the team to build a robust hypothesis and predict an accurate estimate for the output voltage.

2. Signal Processing

The approach for signal processing was simple. An FPGA device was purchased that serves the purpose of a multi-instrument tool with a built-in python API.

[4]For context, the Langevin function describes the probability of finding a given magnetic moment in a particular orientation in response to a magnetic field.

Fig. 5: Moku Go

The feature that was exploited is the waveform generator and spectrum analyser. This provided the coil with a $1.3kHz$ signal. The output signal from the coil was fed back into the Moku Go and the frequency spectrum was found using the spectrum analyser. In essence, much of the signal processing was handled in hardware. The key in the software was to extract the data and locate the peaks of harmonics three and five and after this calculate the ratio. To do so, the approach taken was to use $scipy.signal.find\_peaks(x)$ where x is a 1D array containing the signal data and the output is a dictionary containing the magnitudes of the peaks. The values that represent the 3rd and 5th harmonics are then extracted and the ratio is calculated. Audible frequencies were used to demonstrate change in the ratio. A base frequency of 1300Hz was used to represent the state of the magnetisation response when nothing is detected and other outputs were mapped relative to this frequency.

The code snippet below depicts this:

```
base_frequency = 1300 # Base frequency in Hz
target_frequency = base_frequency * (1/new_ratio)
if new_ratio > 0:
    target_frequency = base_frequency
```

III. Assembly and Testing

a. Amplifiers

The coil requirements meant that the use of amplifiers was going to be needed. A transmit amplifier was used to increase the current of the 1.3KHz sine wave up to 6A. Since the drive frequency sat in the audible range the team was able to purchase an off the shelf audio amplifier. When the magnetisation response left the coil, it was fed back into the Moku Go however the signal was too small to analyse due to the resolution of the ADC built into the Moku Go and ran into quantization errors. To solve this issue the receive signal was amplified to allow the signal to cover the dynamic range of the ADC and extract the highest quality digital representation of the signal.

An amplifier was built (Figure 6) using a simple operational amplifier and two resistors. To test both amplifiers, sinusoidal inputs were used and their amplitudes were noted pre and post- amplification. The Rx amplifier in Figure 6 was assembled and placed into a plastic electronics box that ensured safety and simple usability. A switch and LED light were installed to signal to the user when the amplifier is being powered. 9V batteries were used to power the amplifier for portability reasons hence another reason for the switch. The input to the box was banana cables due to their high

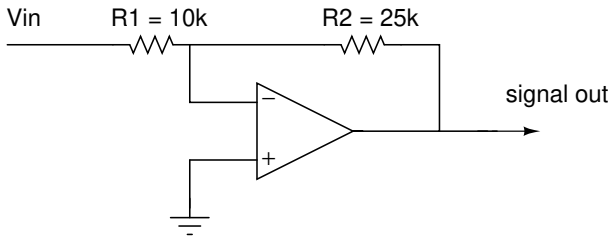

Fig. 6: Rx Amplifier

tolerances for current and voltage and the output of the Rx amplifier is a BNC cable going into the Moku Go. Inside the box was also an ammeter. This served the purpose of tracking the current of the Tx amplifier and preventing the current from exceeding 6A. The gain was controlled by a dial which made it possible to surpass 6A unintentionally, causing inaccuracies in our results and accelerated heating of the coil.

b. Manufacturing The Coil

Once the simulation and de-risking steps were completed, a CAD model was developed. It was driven from a single layout model, itself equation driven with variables. These variables allowed for changes in wire diameter, number of turns, wall thicknesses, etc. to propagate throughout the design and automatically update the casework, coils, and cores. Thereby minimising mistakes, manufacturing of incompatible parts, and repeating work.

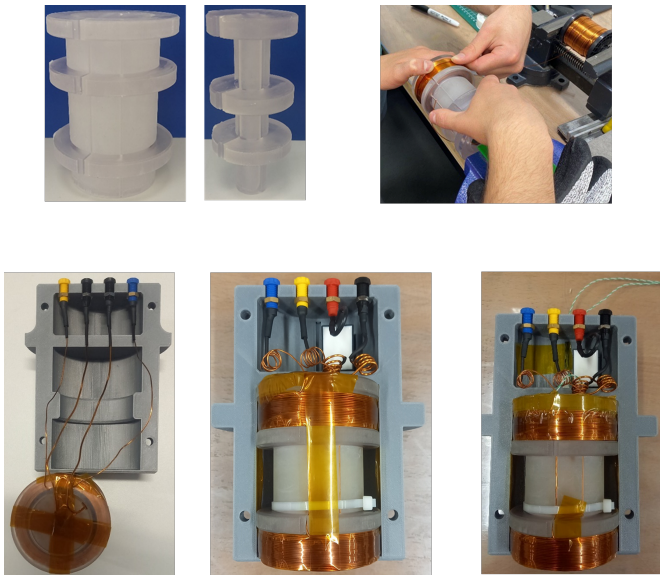

Fig. 7: Coil Manufacturing

It was decided that hand wound, single core and enamelled wire coils were the only feasible solution due to time, wire thickness, quantity, lead time, and cost. The chosen coil-winding method was inspired by mechanical coil winding machines and fishing reels. Figure 7 depicts the coil manufacturing process and integration into the casework.

IV. Results

When testing, the streptavidin dilutions were only prepared when the hardware was ready for testing. This was because its long-term stability in solution was unknown. Certainty was necessary for the biomarker to still be biochemically active. Once combined with nanoparticles the measurements were conducted quickly. A loss in response with time had been observed in the literature (Xiaojuan Zhang, 2013). We followed procedures to extend this response time by adding 0.001% Tween20 (polysorbate 20) to all samples as mentioned in the method section. The sensor was able to detect the presence of the pure nanoparticles (6mg/ml), half the concentration (3mg/ml) and the in-vitro concentration (0.18mg/ml) used in the authoritative paper (Xiaojuan Zhang, 2013). Detection of the in-vivo concentration of nanoparticles (0.002mg/ml) was unsuccessful. Results were measured by observing a change in the ratio between the 3rd and 5th harmonic:

$$R35 = \frac{F(f_3)}{F(f_5)} \tag{10}$$

The results in Figure 8 show the response of nanoparticles for the in-vitro concentration (0.18mg/ml) at eight concentrations of streptavidin and one control. There was a perceptible change in response to the biomarker. Between 200-2000pM the expectation was that the response will be of positive linear correlation, but we observed non-linear fluctuations instead. For clarification, the 3rd and 5th harmonics were always located at 3.9kHz and 6.5kHz. The y was originally on a logarithmic scale in dBm as default for a spectrum analyser however due to the relatively small change between the harmonics it was decided that for visualisation purposes a linear scale was much more digestible.

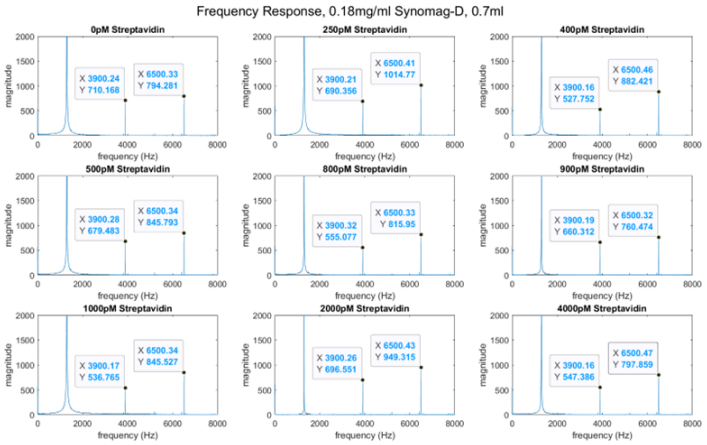

Fig. 8: Results

preliminary test was used to understand the nature of the nanoparticles themselves and provide feedback to the engineers as to how well the coil was built. This test is shown in Figure 9. It was hypothesised that this test will have a higher ratio than the control but a smaller one than the nanoparticle and streptavidin solution and this was indeed the case. Moreover, Figure 10 shows a promising result that nanoparticles can also be detected in a human tissue environment – in this case, simulated by embedding the nanoparticles/streptavidin solution in a hydrogel. This was also tested against a control.

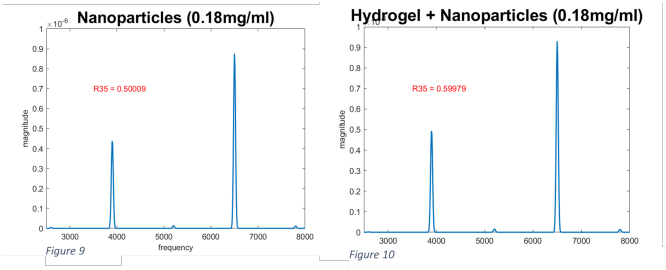

Fig. 9: preliminary tests

V. Discussion

a. Quality and Reliability Of Results

The results in this paper were achieved in 6 months. Given the incredibly short time frame, this is very promising however due to this incredibly short time frame there were not enough iterations of the experiments and therefore data to conclude any consistent trends. The protocol that was developed allowed for results that confidently prove the hypothesis true. For this reason, this first prototyped coil design was an overall success as it allowed the team to gather clear results. Another part of the success was due to the inherent theory behind this technology. The focus on the 3rd and 5th harmonic meant that coil design was much simpler for reasons stated in the coil theory section. However, what is particularly advantageous is the use of the harmonic ratio. Both harmonics have inaccuracies and noise however these inaccuracies are consistent throughout the spectrum and so they cancel out when divided, providing an accurate result. This was not in the literature and therefore more work must be completed to determine how much this theory can be exploited to improve sensing performance. Another area of interest is the trend of the results. The ratio did not show an obvious linear increase with streptavidin concentration which implies that the experiments need to be repeated several times to allow an average to be calculated. An interesting topic that is not discussed thoroughly in the literature is the use of other odd harmonics to extract more useful information from the spectrum. The 3rd and 5th harmonics were chosen in the literature as they have the largest amplitudes and so are most obvious to inspect. However, it sparks questions as to what information could the whole spectrum provide. An idea that is worth exploring is looking at the spectral density of the signal. This would show where most of the energy (and therefore power) of the spectrum is concentrated and may uncover useful information. Another suggestion that is worth exploring is the relationship between the ratios of the 3rd and 5th, 5th and 7th, 7th, and 9th and so on.

b. Improvements

The detection of in vitro concentrations provides confidence that in vivo concentrations can be detected. To do this the coil design will need to improve. The first step in optimising the coil is by winding the coil with a machine. This will ensure that the cancellation part of the coil is subtracting most of the distortion. A hand-wound coil guarantees inaccuracies which are difficult to identify and compensate for. The temperature and cooling of the coil could also be investigated further. It would be useful to model the rate if change of temperature of the coil with time. This would provide a temperature time curve showing the transient and steady state response. It would then be possible to analyse how much magnetic field strength is induced once steady state is reached and whether the magnitude of the field strength is enough to saturate the nanoparticles to provide a valid magnetisation response. 18 If the magnitude is indeed strong enough, then the next step in developing more robust results is calibration. Calibration should be done at steady state temperature of the coil. Since 6A is a large current the expectation is that steady state could damage the coil. To avoid this, the coil can be run in an environmental test chamber where the surrounding temperature is much lower than room temperature, shifting the curve downwards along the temperature axis. When running at steady state record the frequency response when nothing is detected by the coil. Afterwards, conduct the experiments in the same way but also in steady state. The subtraction of the two results would provide a more accurate reading and therefore ratio value. To improve the actual temperature-time curve, cooling would need to be implemented in the next prototype. Since the coil's intended environment is room temperature, a common solution is to install a heat sink and fan. However, the current design would not be suitable for such modifications and therefore an altered design is necessary. This would provide the opportunity to drop the current down slightly more and source better materials which would also improve performance. The size was not a requirement or goal of the project however producing a smaller sized prototype is essential at some point for this technology. As mentioned in the introduction, a Helmholtz coil was initially considered however a coil of that design that generates 10mT would be too large. The decision was made to design a simpler cylindrical coil. There was little time available to consider other coil geometries. Nevertheless, finding a geometry that allows for a smaller form factor and can provide more coil turns is an important next step. The last point of discussion is the software aspect of the project. The role of the software engineers on this project was mainly to process and visualise data therefore it was not inherently challenging or novel. To make full use of the data available, a machine learning model should be considered. It could be trained on data from many nanoparticle + biomarker pairs and concentrations where different biomarkers represent a proxy for different biological processes. The model would be intended to identify what concentration is being assessed and most importantly if a biological event is taken place and if so, how drastic it is. This is challenging as many biological events are notorious for being very difficult to diagnose early however if this can be solved in any domain it would have great potential.

VI. Conclusions

Significant strides have been made towards the detection of nanoparticles and associated biomarkers at in-vitro and in-vivo concentrations. The team has successfully demonstrated the capability to detect nanoparticles at in-vitro concentrations, specifically 0.18 mg/ml (Fe). However, challenges have been encountered in detecting nanoparticles at in-vivo acceptable concentrations. Preliminary investigations suggest that inaccuracies in coil manufacturing may have contributed to this limitation. To improve the sensor, the team

proposes two major design changes: Implement cooling for longer experiment times and calibrate the coil by calculating its rate of change of temperature. The sensor was able to detect the proxy biomarker, streptavidin. However, the response to increasing concentrations did not exhibit a strong trend. This outcome introduces an element of uncertainty and necessitates further investigation and optimization. Looking ahead, the team envisions a future where this technology, fully developed into a robust prototype, has the potential to revolutionize the biotech industry. By addressing the challenges associated with detecting nanoparticles at invivo concentrations and optimizing the sensor's response to biomarker levels, a significant commercial impact is anticipated. The technology holds the promise of transforming the way biomarkers are monitored and analysed in medical devices, thereby opening new frontiers in diagnostics and personalized healthcare.

VII. Acknowledgment

The work described in this report was a collaborative effort between many engineers. The author of this paper took a lead role in building of amplifiers and assisted mathematical modelling and coil theory outlined in this with Roy Kongnyuy. Theo Desbrousses performed further simulations and development described in the coil design section. The majority of the software was developed by David Bolarinwa. Amplifier requirement simulations and component sourcing was conducted by Frederick Watkins. All the content in the biology section was prepared by Morwenna Maunder and Roy Kongnyuy and the actual experiments in the wet lab were conducted by Roy Kongnyuy.

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