Examination of suitable material for phantoms used in photoacoustics

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***Abstract*—A fundamental part of modern medicine is the ability to study and analyze images, especially for diagnostic purposes. A relatively new imaging system is photoacoustics, where laser- and ultrasound technology are combined to create images in a non-invasive way. It is absorption of the laser that is responsible for the creation of the ultrasound signal. Thus, it is the optic properties of tissue that enable differentiating. Normally it is the amplitude of the returning signal which is utilized for image contrasting in photoacoustics. Instead, this study has examined the center frequency to wavelength dependence of the returning signal. Two parameters assumed to have an effect on the center frequency spectrum are the size and colour of particles. The aim of this study was to investigate whether microsphere phantoms can be used to confirm this assumption. Phantoms consisting of microspheres in different sizes in the colours blue, green and transparent were created. The photoacoustic system at Lund University Hospital (VisualSonics Vevo LAZR-X, Toronto, Canada) was used to make nine measurements, during three different days, for each one of these. After analysis of the data from the amplitude of the returning signal, it was concluded that the spheres in blue and green are appropriate for future research in this field. The results from analyzing the mean and standard deviation of the center frequency was that there is a nonlinear correlation between sphere size and center frequency. There was also a distinct difference between the center frequency spectrum for the blue and green spheres.**

I. Introduction

Medical imaging is an important part of our healthcare system, particularly in areas of diagnostics. Increasingly more advanced technologies have made it possible to create high resolution models of human anatomy and biological processes. By studying these images diagnosis, treatment monitoring and disease outcome predictions can be made [1]. A relatively new player in the field is photoacoustic imaging. This is a technique which uses a combination of laser light (photo) and ultrasound (acoustics) to produce images. Laser pulses are emitted from laser fibers and as the light passes through matter, part of the energy will be absorbed and transformed into heat. This results in a thermal expansion of the absorbing particles. In turn, pressure waves are created which propagate through the surrounding matter. These waves can then be detected by a traditional ultrasound transducer.

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A. Photoacoustic imaging

In the case of ultrasound it is the difference in acoustic impedance of a material which is responsible for signal differentiation. The main difference between photoacoustic in comparison with traditional ultrasound imaging is that, rather than mechanical properties, it is the optical properties that define image contrasts. Depending on the optical absorption of the biomolecules within the tissue, in respect to the excitation wavelength, the thermal expansion and corresponding pressure distribution will vary. By constantly measuring time differences between emitted light and acoustic wave detection the depth and location of absorbing tissue can be calculated and an image be created. It is this difference in how contrast is created that makes photoacoustics superior to ultrasound in certain respects [2]. Optical absorption is generally more selective than the mechanical properties of a material. Photoacoustics also allows for both anatomical and functional imaging, something which is not possible with ultrasound [3]. Further benefits include the fact that the light is non-ionising, contrary to several other imaging systems. Also, due to the fact that the structural and operational requirements are not very complex and the system is relatively inexpensive, it is accessible for clinics with smaller resources [3, 4]. As a result of high costs and high training requirements, other imaging systems are not readily available everywhere in the world. Photoacoustic imaging could potentially become an effective way to strengthen the global situation in this area. However, due to high absorption and scattering effects of tissue photoacoustics has a lower penetration depth than ultrasound alone [2].

B. Research prospects

Photoacoustics is commonly used in clinical studies to image blood vessels and measure oxygenation levels [2]. The low penetration depth makes the technology unsuitable for deep body applications. However, it is well adapted for small depth conditions and at Lund University research is mainly being made on the potential for the equipment to be used as a tool for skin cancer diagnostics. The technology is still in an exploratory stage and its full potential is yet to be discovered. For any wider use of photoacoustic imaging to become a reality more research must be made. Increased understanding of how different molecules to being irradiated by the laser would provide fundamental information in the field. This would allow for more standardised utilisation and an expansion of potential applications in the future. For example, with greater knowledge of absorption patterns and corresponding ultrasound frequency spectrums, new and more specific biomarkers could be used to create more accurate differentiation between healthy and diseased tissue.

C. Center frequency

Today, it is only the amplitude of the returning signal which is actually used for image analysis. Few studies have been made on how the frequency of the returning ultrasound wave could be used for differentiation between different types of tissues and structures. Two examples are *Characterization of bone microstructure using photoacoustic spectrum analysis* by Feng et al. [5] and *Frequency-domain analysis of photoacoustic imaging data from prostate adenocarcinoma tumors in a murine model* by Kumon et al. [6], where the spectral density is studied in the frequency domain. However, neither of these are looking at the center frequency spectrum of the signal. When measuring, the photoacoustic system will register a band of returning frequencies for each wavelength that is being sent out by the laser. The frequency at the center of this band is called the center frequency. Thus, the center frequency spectrum shows the relationship between emitted wavelength and returning ultrasound frequency. It is this relationship, which is previously unexplored, that will be the focus of this study. Henceforth, the terms "frequency" and "center frequency" will be used interchangeably throughout the report. Knowledge about this relationship could open up for additional material characteristics, giving us new ways of differentiating between tissues in image development.

D. Thesis

The purpose of this study was to examine the photoacoustic response (primarily the frequency) from microspheres of different parameters, namely colour and size. The perceived colour of an object is defined by the light absorbing chromophores within the molecules [7]. In theory there should be a frequency dependence correlated to size and colour, but no actual studies have been made to confirm this before. Foremost, investigations must also be made into whether the chosen material is at all suitable for this type of experiment. The effect of the microsphere concentration will also be examined.

E. Agenda

Photoacoustic measurements will be made on phantoms containing plastic microspheres. By varying size and colour of the spheres and comparing the resulting spectrums potential differences or similarities in the frequency response will be identified and evaluated.

II. Method

A. Material

- Polyethylene microspheres (Cospheric, Santa Barbara, CA, USA. See table I)
- Paraffin oil (mineral oil), TRIKEM VET, Malmö, Sweden

B. Equipment

- Photoacoustic imaging system (VisualSonics Vevo LAZR-X (transducer MX400), Toronto, Canada)
- Pipett with 0-1000 ml tips
- VWR Cuvettes PMMA (VWR, macro, Leuven, Belgium)
- Scales
- Safety glasses
- Disposable gloves
- MATLAB (Mathworks, Massachusetts, USA)

Table I: List of microspheres used in this study

"Name"	Colour	Size (μm)
Blue 75	Blue	75-90
Blue 53	Blue	53-63
Blue 38	Blue	38-45
Green 75	Green	75-90
Transparent 75	Transparent	75-90
Transparent 53	Transparent	53-63
Transparent 38	Transparent	38-45

The blue and green colours were picked due to their appropriate absorption range within the EM-spectrum for the emitted photoacoustic laser light between 680-970 nm. The transparent spheres were selected for evaluation of whether it is the colour of the spheres that induce light absorption rather than some other material factor.

C. Workflow

An initial trial was made to ensure that the cuvettes, oil and spheres were suitable for use with the photoacoustic system. No significant photoacoustic signal was detected from pure mineral oil within the cuvette. After having added spheres to the cuvette a signal was detected and so the equipment could be deemed appropriate for the purpose of the project.

A phantom was created by mixing 100 parts of mineral oil with one part microspheres in a glass beaker. The proportions were measured by mass. The phantom mixture was then transferred to a cuvette with a pipette. By pipetting 1:1, 1:2, 1:4 ratios of the original phantom mixture with pure mineral oil two further microsphere concentrations were created. This process was repeated for all sizes and colours of microspheres. Thus, three cuvettes; 1%, 0.50% and 0.25% microsphere to oil mass ratio, were made for each type of microsphere. Henceforward, when mentioning a cuvette, for example Blue 75, it will be in reference to the 1% concentration if nothing else is stated. Two cuvettes were filled with pure mineral oil. The same cuvettes and phantom mixtures were used throughout the project.

The first photoacoustic measurements were done on the same day as the phantoms were made. The spheres had a tendency to aggregate and stick to the sides of the cuvette, probably due to their triboelectric nature. This phenomenon can be seen in figure 1. To prevent this and ensure a homogeneous distribution during measurements, the phantoms had to be vigorously shaken. The shaking had to be done in close proximity to measuring as clear signs of aggregation could be seen within seconds to minutes of mixing. For each phantom two consecutive measurements were made, re-shaking the cuvettes and re-adjusting the ultrasound transducer between.

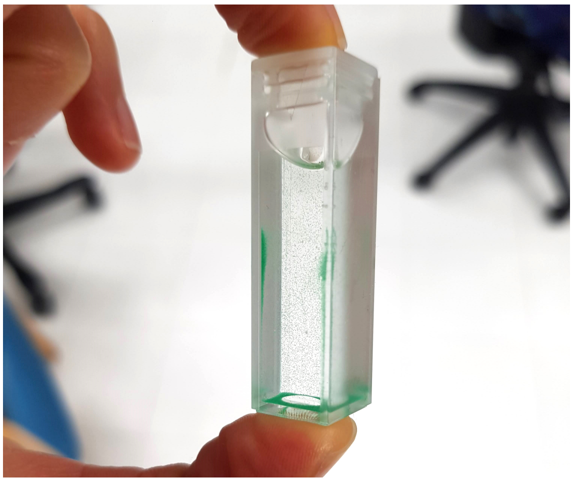

Figure 1: Picture of the phantom for Green 75 at 0.25% mass concentration. Signs of clear signs of microsphere aggregation can be seen at the walls of the cuvette.

All the data from the photoacoustic measurements was transferred to MATLAB where amplitude-to-wavelength as well as frequency-to-wavelength spectrums were created. Based on an initial analysis of these first measurements, decisions where made on how to proceed with the project and further measurements.

It was then decided to only continue with the blue and green microspheres, and with one cuvette of the pure mineral oil. Since the transparent microspheres did not produce any photoacoustic signal, the frequency spectrum consisted only of background and there was no point in doing further measurements on these.

Three additional sessions were performed in order to collect data for analysis. One day before the first of these three sessions the cuvettes were placed lying down to allow for the spheres to settle at the bottom wall of the cuvette. The purpose of this was to study what the potential effects might be on the photoacoustic signal due to particle aggregation. First, three consecutive measurements were made, with re-adjustment of the transducer but without any shaking in between, for each cuvette. Next, three more measurements were made for each cuvette, but this time they were shaken to create a homogeneous distribution of the spheres within the cuvettes. The measurements on the shaken cuvettes were done for each phantom one at the time and then looped three times. Before all the measurements it was necessary to make sure that the transducer was perpendicular to the cuvette, which can be seen in figure 3 as the yellow line is perpendicular to the axis. The setup for the measurements can be seen in figure 2.

The same procedure was followed for the next two sessions which were done the same week. All of this was done several weeks after the initial creation of the phantoms.

Measurements on the cuvette with the pure mineral oil were made only two times per session. First, one time without shaking and then one time after having been shaken. This was done in order to investigate if the air bubbles created whilst shaking would effect the signal. The pure, non-shaken oil was also used to ensure that the oil and cuvette themselves did not create any significant signal.

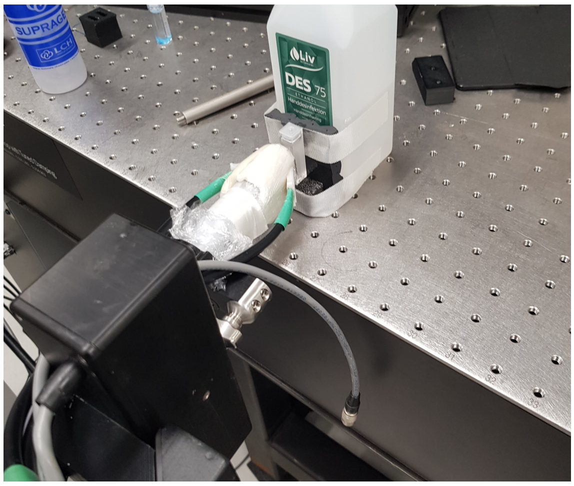

Figure 2: Picture of phantom and photoacoustic probe setup during measurements.

All the collected data was transferred to MATLAB to be analyzed. Firstly, choosing an appropriate Region of Interest (ROI) for examination of the amplitude and center frequency. In figure 3 and figure 4 the photo acoustic image of the absorption from the shaken and non-shaken cuvettes is visualized and the appropriate ROI can be seen represented by the black rectangle. The measurements from the three session were analyzed for each cuvette by looking at the mean and standard deviation from all nine measurements.

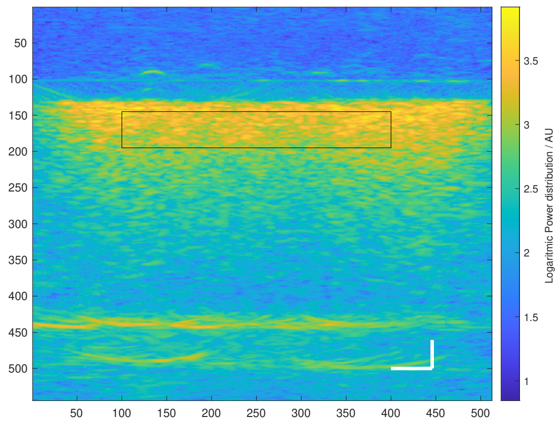

Figure 3: Appropriate ROI for a shaken cuvette in the photoacoustic image. The numbers on the y- and x-axis represent the number of pixels in the image. The white lines represent 1 mm in the x-direction and y-direction respectively.

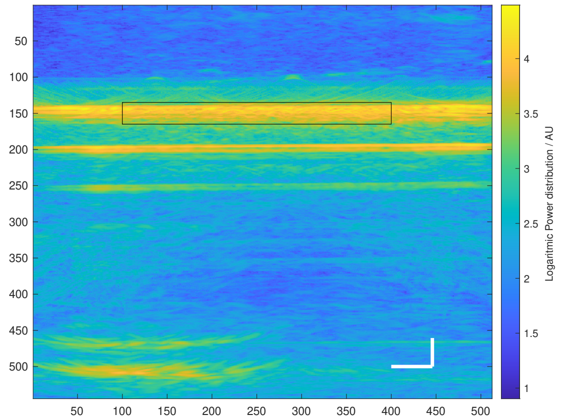

Figure 4: Appropriate ROI for a non-shaken cuvette in the photoacoustic image. The numbers on the y- and x-axis represent the number of pixels in the image. The white lines represent 1 mm in the x-direction and y-direction respectively. The yellow lines below the topmost are a result of signal echos in the cuvette wall.

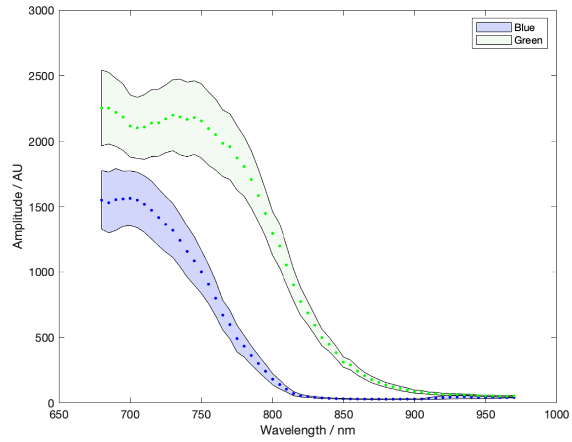

Figure 5: The dotted lines represent the mean and the shaded area the standard deviation for the amplitude of all nine measurements for Blue 75 and Green 75, shaken cuvettes. After approximately 800 nm and 900 nm there is almost no signal left for each colour respectively.

D. Safety

Laser safety glasses were used during measurements. Gloves were worn while handling the cuvettes and microspheres to avoid unnecessary skin contact. All waste was thrown in the bin to avoid micro-plastics ending up in the drain.

III. Results

In figure 5 the amplitude spectrum of the photoacoustic signal is visualized for Blue 75 and Green 75. As seen the amplitude for the signal from green has a larger range, compared to blue. Furthermore, it can be seen that the signal is significantly lowered after 800 nm for blue and 850 nm for green. For this photoacoustic system, amplitude signals below 100-200 AU can be considered as background. Thus, the received center frequency signal after this wavelength threshold can also be neglected as a product of noise.

A. Sphere size

Figure 6 shows that there is a difference in frequency spectrum between the three different sizes. The largest spheres (Blue 75) have the highest center frequency whilst the middle sized spheres (Blue 53) have the lowest. Thus, the smallest spheres (Blue 38) have a lower center frequency than Blue 75 but still higher than Blue 53 during the major parts of the spectrum. There is a significant difference in the spectrum for the largest spheres compared to the two other sizes. Whilst the difference in center frequencies between Blue 53 and Blue 38 is not as large as for Blue 75, there is almost no overlap in standard deviation. Thus, they are clearly not the same.

B. Sphere colour

Looking at figure 7 one can see that there is a difference in shape between the spectrums of center frequency for Blue 75 and Green 75, but they almost start at a similar value.

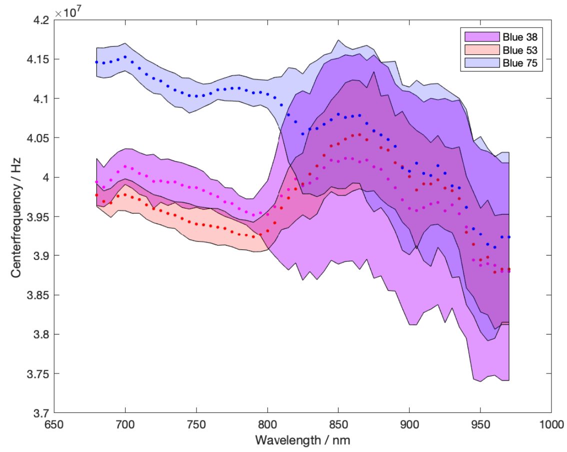

Figure 6: The dotted lines represent the mean and the shaded area the standard deviation for the center frequency of all nine measurements for Blue 38, Blue 53 and Blue 75, shaken cuvettes. After 800 nm the signal is mostly background and noise.

C. Sphere concentration

The frequency spectrums for the three different concentrations are almost entirely overlapping in all four types of spheres. This can be seen, represented by the results for Blue 75, in figure 8.

D. Non-shaken cuvettes

In figure 9 the frequency spectrum for Blue 75 is visualized for both the shaken cuvettes and the cuvettes that were not shaken. At first sight the large difference in standard deviation is noted. Moreover, both the shape and position of the spectrums are different between the two. This was the general case when

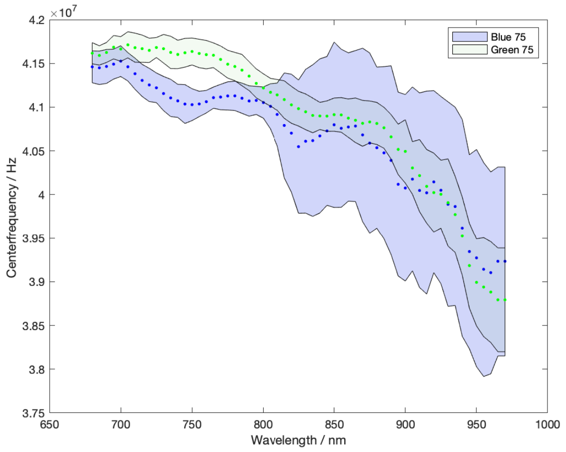

Figure 7: The dotted lines represent the mean and the shaded area the standard deviation for the center frequency of all nine measurements for Blue 75 and Green 75, shaken cuvettes. For Blue 75 the signal is mostly background and noise after 800 nm. For Green 75 the background and noise starts around 850 nm.

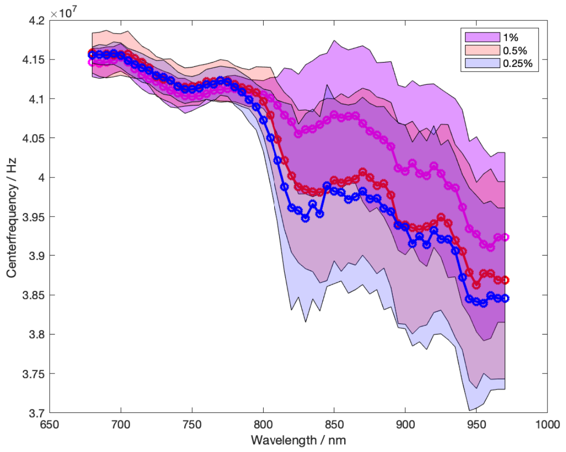

Figure 8: The dotted lines represent the mean and the shaded area the standard deviation for the center frequency of all nine measurements for the different concentrations (1%, 0.50% and 0.25%) of Blue 75, shaken cuvettes. After 800 nm the signal is mostly background and noise. The figure looks very messy due to the high degree of overlapping of the mean and standard deviation between the different concentrations.

comparing the results for the shaken and the non-shaken cuvettes.

E. Pure mineral oil

The amplitude spectrum for the oil, both shaken and non-shaken, is illustrated in figure 10. The absorption spectrum and frequency spectrum for the pure oil show that neither the cuvette with or without air bubbles created any significant signal. One can see that the amplitude, and thus the signal, is

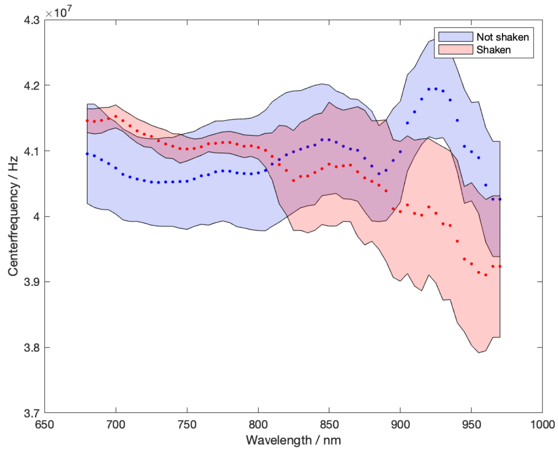

Figure 9: The dotted lines represent the mean and the shaded area the standard deviation for center frequency of all nine measurements for Blue 75, shaken and non-shaken. After 800 nm the signal is mostly background and noise.

significantly lower than in figure 5. It can also be seen that the air bubbles do not induce an increase in signal.

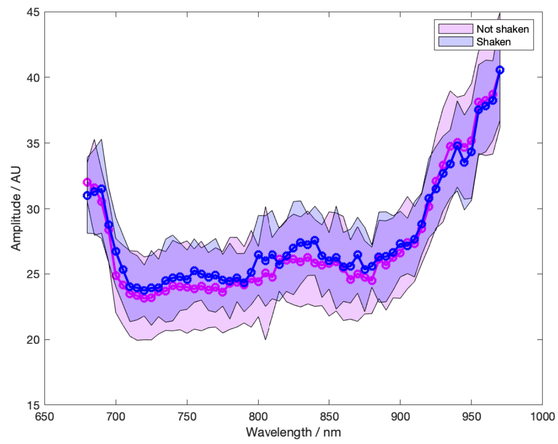

Figure 10: Amplitude spectrum for shaken and non-shaken pure minereal oil, where the dotted line is the mean and the shade is the standard deviation.

IV. Discussion

A. Reproducibility

As always, there is an uncertainty in the results of a single measurement and considering the fact that this is an area of research which is previously unexplored there could be no certainties of how different parameters would or would not affect the results of the measurements. Additionally, there is no previous reference of what one would expect the resulting frequency spectrums to look like. An important part of this study has therefore been to evaluate and minimise the effect of such uncertainties in the results. As stated in the method some experiments had to be done on order for a clear workflow to be created. The aim was to increase credibility through performing a large amount of measurements. This created more data to analyse and a means of verifying the reproducibility of the study. In total, nine measurements were made for each cuvette that had been created. These nine measurements were conducted over three different sessions on three different days. As one can see in the figures the outcome of this work looks promising. The standard deviation for the shaken cuvettes is relatively small and uniform over the relevant spectrum range. One can therefore conclude that the results must be quite reliable and the reproducibility of the method that was used to be good.

It is obvious that the standard deviation seems to increase considerably above around 800 nm for the blue spheres and around 850 nm for the green ones. However, this is to be expected since, as mentioned in the results section, those are the wavelengths where the microspheres are not absorbing the light emitted by the laser. All the perceived signal above this wavelength will only be a product of random noise and the weak background, which inevitably leads to a higher standard deviation.

B. Size correlation

Looking at figure 6 one can see that there is a difference in characteristic shape, in respect to wavelength dependence, of the center frequency spectrum between the different sizes of microspheres. Thus, this could potentially be a particle characteristic relevant for future photoacoustic applications.

Comparing the three spectrums between the three different sizes it appears that the center frequency correlation is non-linear as in figure 7, 8. This is somewhat surprising as one might expect some kind of increasing or decreasing relationship between the size of the sphere and the level of the frequency response. The largest spheres do have the highest frequency response, but it is the middle size that has the lowest frequency response. Meanwhile the smallest sized spheres display frequencies in the middle of the two previously mentioned. It would be interesting to further investigate the reason behind this phenomena.

C. Colour correlation

When instead looking at the difference in spectrum in respect to colour, as can be seen in figure 7, it can once again be seen that there is a difference in the general shape of the frequency spectrum. There is not, however, a very clear distinction in the level of the center frequency. One reason for this could potentially be that green and blue are too close to each other in the EM-spectrum for us to be able to detect any obvious difference. At least not without having any inclination of how large a ”significant difference” could be expected to be.

D. Non-shaken cuvettes

As mentioned in the method there were some issues during testing that the spheres were quick to aggregate, making it slightly difficult to ensure a homogeneous distribution of the spheres within the oil. It also created an additional question of issue; would the spheres still act as individual particles when exposed to the laser, even in an aggregated state, or would each cluster act as a new particle of a larger size. In an attempt to study this, measurements were made on particles that had settled onto the wall of the cuvette, as described in the method. The results from these measurements were surprising and also harder to analyse. One very clear difference between these compared to the cuvettes that had been shaken was that the standard deviation was considerably larger for all these measurements. This can be seen represented by Blue 75 in figure 9. A theory could be that the layer of spheres created on the wall have arranged themselves into clusters of different sizes. As previously discussed there seems to be a correlation between sphere size and frequency spectrum. So, if there in this situation is a large, and perhaps irregular, range of cluster sizes in the same measurement one could expect a larger variation in the corresponding frequency response from this measurement. Thus, resulting in a greater standard deviation.

E. Concentration dependence

By comparing the spectrums within the relevant wavelength range, as seen in figure 8, one can conclude that the concentration does not seem to have any notable impact on the resulting frequency spectrum. The fact that all three measurements are so similar for the three cuvettes with the same type of sphere actually strengthens the reliability of the results.

F. Clinical significance

The results show that size and colour seems to have an effect on the center frequency spectrum of the photoacoustic signal. Different types of tissue includes different types of chromophores and can be assumed to consist of different effective sizes of structures. After learning more about the correlation between these parameters, the frequency response and of how to interpret the spectrums, these could potentially be used for tissue differentiation. Disease diagnostics or creation of medical images are examples of how this knowledge could then be used in a clinical setting.

G. Future

Having been a pioneering study within the field of photoacoustics there have been a lot of new questions created along the way that have been left unanswered in this study. The issue of the aggregating spheres created some problems that simply had to be accepted as time and resources were not sufficient for any better solutions to be found. For future research however one might investigate whether there is a more effective and reliable way of ensuring homogeneity during measurements. Perhaps one could mould the spheres within Styrene-ethylene-butylene-styrene (SEBS) or mix them in another way than shaking. Shaking the cuvettes inevitably invited air bubbles to the oil. Whilst the bubbles themselves did not appear to create any photoacoustic response the effect they might have on the distribution and expansion of the spheres is unknown and might have impacted the results.

In this study only three different sizes of spheres for a single colour (blue) and only one other colour (green) were used. It would be interesting to examine a larger range of sizes and colours to get a better idea of how the center frequency correlates to size and whether this correlation is consistent between different colours or not. As mentioned, our chosen colours, blue and green, are next to each other in the EM-spectrum. Perhaps this results in only a small difference in signal.

It would also be interesting to conduct optical measurements on the transparent spheres to investigate the optical properties of the spheres themselves. This could provide valuable insights which would lead to increased understanding of how the spheres react when exposed to the laser.

H. Ethics and Sustainable Development

One of the major concerns for this experiment regarding sustainable development is the use of microspheres in the material polyethylene (plastic). One of the seventeen Global Goal is 14: Life Below Water, where the concern for micro plastics in the ocean is rising [8]. Carefulness when handling the microspheres is important to reduce the disposal into lakes, oceans and other waters. Ingestion of micro-plastics is alarming since the material is related to impact in human health [9]. For example, evidence has been presented that micro plastics have been found in human placenta [10]. Important to keep in mind that the volume of micro plastics used in this kind of experiment is insignificantly compared to industrial and consumer pollution.

One positive aspect of photoacoustics is the noninvasive procedure, that can lead to better diagnostics in countries that are lacking safety and sanitary measures in the medical field. This aspect is related to Global Goal 3: Good Health and Well-being [11].

It has been shown that photoacoustics has a different effect on darker skin than lighter skin. Affects such as higher signal from the skin surface and lower penetration. Thus, photoacoustics does not work as well on darker skin and this could lead to inequality in medical treatment [12]. This problem should not slow down the research on photoacoustics but should be kept in mind when developing the technique further.

V. Conclusion

The results clearly show that the spheres that were used in this study are suitable for photoacoustic measurements in the respect that they do create photoacoustic signals. However, there is a downside to the aggregating properties of the spheres that complicates the experiment and compromises the credibility of the results slightly. Nevertheless, the spheres were sufficient to produce interesting results and from these the conclusion can be made that at least the size seems to have an impact on the frequency spectrum for that particle. The colour also seems to have a certain effect, although slightly smaller than that of the size. The fact that these differences can be seen implicates that center frequency spectrums could be used for clinical applications in the future by differentiating particles based on their size or the chromophores they carry. However, before this knowledge can be exploited in practise further studies and closer analysis of the nature of the relationship between size/colour and frequency response must be made.

VI. Acknowledgements

The main supervisor for this study has been Tobias Erlöv. He is experienced in the research field of photoacoustics and has been a driving force in the formulation and execution of the project.

Azin Khodaverdi is a doctoral student and have been working alongside Tobias with the photoacoustic measurments. Azin is mainly responsible for the development of the Matlab code that has been used for processing the raw photoacoustic data.

With her many years of experience in experimental physics and optics, Nina Reistad has provided the project with a lot of valuable insights and has helped interpret and work around some of the issues and questions that have come up along the way.

A special mention must be made for John Albinsson who has been present during all laboratory work and acted as our technical supervisor whilst using the photoacoustic equipment. With a lot of experience of the practical operation of the machine he has overseen that everything has been done correctly and appropriately.

The person mainly responsible over this project has been Magnus Cinthio who has been present along the way for input into the proceedings and results.

Between the two authors all of the laboratory work has been done together. Malin has been more involved in the writing of code in MATLAB for data processing and presentation whilst Klara has been working more on the writing of this report. All the work has been co-reviewed and a dialogue has been present throughout the project.

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