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# DEVELOPMENT OF A PHOTOACOUSTIC INSTRUMENT FOR THE DETERMINATION OF THE PHOTOTHERMAL PROPERTIES OF NANOPARTICLES

A Thesis in

Chemistry

by

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#### ABSTRACT

Chemical reactions that typically require heating of a bulk solution in order to proceed are one recent target of the push towards greener chemical methods. One method employed to reduce the amount of energy input needed for such reactions is to use the photothermal effect in nanoparticles (NPs) to heat the reactant molecules locally without heating the whole reaction mixture. Determining the amount of thermal energy produced by NPs is of concern in order to maximize the efficiency of such methods, but most approaches to date only indirectly report on this heat by monitoring the progress of the target reaction.

I used photoacoustic (PA) analysis to measure the heat produced by NPs, specifically dodecanethiol-functionalized gold nanoparticles (AuNPs) and oleylamine-functionalized iron oxide nanoparticles (Fe<sub>3</sub>O<sub>4</sub> NPs). PA analytical techniques use microphones or vibration-sensitive piezoelectric detectors to acoustically measure the pressure front caused by the propagation of thermal energy away from an analyte. For such measurements, thermal energy is generated by the non-radiative (thermal) relaxation pathways in the analyte of interest.

Using a frequency-doubled pulsed Nd:YAG laser to irradiate the NPs, a piezoelectric detector mounted to a 1 cm pathlength cuvette, and a 1 GHz digital oscilloscope for signal processing, a simple, custom-built, and adaptable photoacoustic cell and measurement technique was developed to measure the acoustic pressure signal produced by nanoparticles. The dependence of the photoacoustic signal strength upon laser energy and nanoparticle concentration has been tested, revealing a linear dependence upon each within limits of optical density and laser energy. Preliminary efforts have been made to calculate the heat flux and temperature changes that produced the photoacoustic signal.

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## LIST OF ABBREVIATIONS AND VARIABLES

NP	Nanoparticle
PA	Photoacoustic
PAS	Photoacoustic Spectroscopy
AuNP	Gold nanoparticle
Fe <sub>3</sub> O <sub>4</sub> NP	Iron oxide nanoparticle
UV-Vis	Ultraviolet-Visible spectroscopy
Nd:YAG	Neodymium:yttrium-aluminum-garnet

# <u>Units</u>

P <sub>A</sub>	Photoacoustic pressurePa
<i>k</i>	Instrument constant Dimensionless
r1	Distance from laser beam center to detector m
r2	Distance from beam center to beam edge m
E <sub>o</sub>	Irradiation energyJ
3	Extinction coefficient of NPs at 532 nm $m^{-1}(m/m \%)^{-1}$
β	Volumetric expansion coefficient of the solvent $K^{-1}$
ν	Speed of sound in the solventm/s
C <sub>p</sub>	Heat capacity of the solventJ/(mol*K)
R	Radius of the laser beam m
ΔV	Change in volume of the solventL
β <sub>T</sub>	Isothermal expansion coefficient Pa <sup>-1</sup>
ΔΤ	Change in temperature of the solventK
Q/np	Heat released per nanoparticleW/np
n <sub>solv</sub>	Moles of solvent in the laser beam volume mol
N <sub>np</sub>	Number of NPs in laser beamnanoparticles
T <sub>RT</sub>	Room TemperatureK
σ <sub>sb</sub>	Stefan-Boltzmann constant $W/(m^2 * K^4)$
A	Surface area of a NP m <sup>2</sup>
$\epsilon_{np}$ ' and $\epsilon_{np}$ "	Dielectric constant of the NP material Dimensionless
r <sub>m</sub>	Refractive index of a mediumDimensionless

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#### Chapter 1

# Heat Production by Nanoparticles and Photoacoustic Spectroscopy

#### Section 1.1 Heat Production by Nanoparticles

Research in chemistry is taking a turn for the energy-efficient. In particular, the generation of heat in chemical reactions is of major importance as it is the most common tool for overcoming reaction barriers and needs to be dealt with in exothermic reactions. Typically, when heat is needed to drive reactions, the bulk solution is heated to a given temperature, even though the energy required to do this is often far in excess of that utilized by the reactant molecules.

One approach to reducing the required heat is to use a catalyst. The use of heterogeneous gold catalysts has already led to lower temperature oxidation reactions [1], while nanolithography has been achieved using gold nanoparticles. [2]

Providing energy on a scale more relevant to the reactant molecules is another route to greener, and even more finely controlled chemical reactions. One way to do so is to embed nanoparticles (NPs) around them and generate heat *via* the photothermal effect in the NPs. The photothermal effect arises from the non-radiative relaxation pathways through which absorbed light energy is released. While most materials exhibit a photothermal response to some degree, it is more significant in materials which do not fluoresce well, including many types of nanoparticles (NPs). The heating in NPs arises from the transfer of absorbed light energy from the electrons to phonon modes in the lattice of the NP as the hot electrons relax. [3] If the wavelength of excitation is longer than the diameter of the NP, the heating is uniform throughout the NP and is then dissipated to the surroundings. [4]

Metal NPs are of particular interest as they contain sharply absorbing surface plasmons. [3, 5] The surface plasmon arises from the oscillation of the surface electrons in a nanoscale noble metal following absorption of light energy that is resonant with the oscillation frequency. [3, 6] Achieving surface plasmon resonance results in an enhancement of the electric field surrounding the NP, thereby enhancing absorption, scattering, and heat production. While the potential compositions of plasmon-containing NPs are varied, the present work began the investigation of the photothermal effect in NPs by examining spherical gold nanoparticles (AuNPs), which exhibit a plasmonic resonance that overlaps with the second harmonic of a Nd:YAG laser [7], used for the work presented in this thesis.

The photothermal effect in AuNPs is already utilized in a wide variety of biological applications. [8] Gold nanocages can be used to target, image, and subsequently thermally ablate breast cancer cells if they are tuned to mid-infrared light, where body tissue is transparent. [9-15] Dubbed Plasmonic Photothermal Therapy (PPTT), this branch of oncological treatment offers an alternative to other kinds of radiation therapy and chemotherapy, and has seen reasonable success in mice with a potential penetration depth of over 3 cm into body tissue. [16] The success of the photothermal effect of gold in biology provides hope that they will prove useful in chemistry as well. However to fully exploit them we must understand the heat that they produce.

Section 1.2 Application of Photoacoustic Spectroscopy to Nanoparticles

In determining the photothermal properties of NPs, an analytical technique is required which is not impacted by the scattering of light. Photoacoustic spectroscopy (PAS) is one such technique and is the study of the absorptive properties of substances as they pertain to the conversion of light energy into thermal energy. The physical process the technique is based upon dates back to the 1880s when Alexander Graham Bell discovered the "photoacoustic phenomenon" while working on the telephone. [17] The photoacoustic phenomenon was largely ignored as a source of information for analytical techniques until the 1970s when lasers became common in scientific laboratories. Rosencwaig and Gersho did much of the pioneering work on the technique then, and they adapted it for use on gasses, liquids, and solids. [18] Often their experiments utilized inhouse built sample holding and detecting equipment.

Perhaps the most useful aspect of PAS is that it can analyze not just liquids, solids, and gasses, but also highly scattering and porous samples. The PAS signal is developed from absorption of photons and is therefore not falsely enhanced or diminished from scattered light. The end result of this is that the coefficient measured via PAS is truly sensitive to only absorption, not extinction due to both absorption and scattering. In applications where photothermal processes arising from absorption are of concern, being able to easily differentiate between the two is crucial.

The use of PA methods on NPs is not without precedent. Biological imaging with tomography benefits from PA techniques by enabling the selective imaging of tissues or cells which are selectively sought out by NPs. [19] The detector in PA Tomography (PAT) is a microphone or piezoelectric device which is moved around the target tissue to produce a 2 or 3-dimensional image. [16] These biological studies with PAT use the intensity of the PA signal to produce an image, however they rarely quantify the PA intensity in terms of pressure or energy [16, 20], although the technique can be used to measure the optical properties of NPs. [21] However there is relatively little work on photothermal effects and PAS on NPs with an eye towards chemical applications.

#### Section 1.3 Thesis Overview

The components, assembly, and design considerations of the instrument itself are presented in Chapter 2. Here a description of the typical data obtained with this instrument, along with justifications for the various components used and troubleshooting attempted will be covered. The equations governing photoacoustic signals depend heavily on the arrangement of the instrument and are covered next in Chapter 3. The first analyte measured with this instrument was gold nanoparticles (AuNPs), and the PA data obtained using them, along with supporting data and instrument properties calculated using the data, is covered in Chapter 4. Iron oxide (magnetite) nanoparticles (Fe<sub>3</sub>O<sub>4</sub> NPs were utilized after the AuNP studies concluded, which resulted in the data discussed in Chapter 5. Again supporting data and instrument constant calculation results are presented here. Chapter 6 uses the results of Chs. 4 and 5 to attempt to quantitatively determine the photothermal heat that produced the signal and the associated changes in temperature. Chapter 7 summarizes this work and proposes future directions.

#### Chapter 2

## **Development of the Photoacoustic Instrument**

#### Section 2.1 Introduction and Instrument Schematic

Determination of the photothermal properties of nanoparticles first requires an instrument capable of measuring them. PA measurements often require specific analytical conditions, which become rather expensive to achieve with commercially available equipment. Therefore many PA instruments are constructed in-house. So the first steps of this work were to construct an instrument for visible measurements. This chapter details the design considerations and practical issues involved in construction of a working photoacoustic instrument.

The first step in constructing a PA instrument is to select appropriate components. The photoacoustic (PA) signal is susceptible to acoustic and vibration noise in the laboratory due to the nature of the microphone or piezoelectric detector. The signal detector must therefore be able to detect the desired signal while omitting much of the acoustic noise present in a laboratory. The detector must be easily attached to the sample holder, which must be transparent to the wavelength of light used to excite the analyte. The sample holder should also be easy to clean and alter in order to accept different sample types.

The signal processor in PA spectroscopy should be selected such that it can be triggered by the light modulating mechanism. It should also permit routine data exportation for further analysis.

A schematic diagram displaying components that satisfy the above conditions and their connectivity is presented in Figure 2-1, while Figure 2-2 presents images of these components. The specific components used were a Nd:YAG laser outputting 532 nm light, a photodiode arranged orthogonal and adjacent to the laser beam path, the sample cell in the beam path, a preamplifier, and an oscilloscope. The detector was connected to the oscilloscope through the preamplifier using BNC cables, and the photodiode was connected to the oscilloscope directly with BNC cables. The photodiode is arranged such that it is illuminated by the scattered photons of the passing laser beam without the need of a beamsplitter directing the beam directly onto the photodiode (Fig. 2-2a). Irises are also pictured that were used to aid in directing the laser beam onto the sample cell (Fig 2-**2a**). In front of the detector the cuvette of the sample cell can be seen while it is being illuminated (Fig 2-2b). The control module for the laser is also pictured, displaying the analog knobs used for adjustment of laser energy and q-switch delay, the latter of which was adjusted so as to maximize output energy (Fig 2-2c). The signal processing device, the digital oscilloscope, is also pictured showing the sample collection mode and the Tjunctions used to terminate the BNC cables (Fig 2-2d).



Figure 2-1: Schematic of the PA instrument. a - Nd: YAG laser, b - laser beam path, c - photodiode, d - sample holder and piezoelectric detector, <math>e - preamplifier, f - oscilloscope.

With this overview in mind, the following sections discuss the selection and assembly of the components (Section 2.2), obstacles and considerations that improved the system (Section 2.3), and conclusions drawn from the work (Section 2.4).



Figure 2-2: Key components of the PA instrument. (a) The metal post holding the irises and sample cell holder on the same optical axis, (b) the sample cell holder containing a gold nanoparticle solution in toluene being irradiated with 532 nm light, (c) the external controller for the Spectra Physics Quanta Ray 130 Nd:YAG laser, (d) and the Tektronix 4104B DPO in measurement mode with Channels 1 and 4 receiving the photodiode trigger and amplified piezoelectric signal, respectively.

#### Section 2.2.1 Light Source

The photoacoustic signal is dependent upon the amount of light energy absorbed, and absorbance is in turn dependent upon the incident light energy. The light source chosen must therefore produce a photon fluence that enables adequate absorption for a strong photoacoustic signal to be produced while simultaneously not being so strong that it destroys the analyte, as that would defeat a key benefit of PA analysis. Modulation of the light will be required and performed such that the light is either completely attenuated or switched off in between periods of irradiation. Given these considerations, a monochromatic Spectra Physics Quanta Ray 130 Neodymium: Yttrium-Aluminum-Garnet (Nd:YAG) pulsed laser was used for the light source. The principle output of the Nd:YAG is capable of reaching 400 mJ/cm<sup>2</sup> at 1064 nm, with a second harmonic output of 200 mJ/cm<sup>2</sup> at 532 nm. The second harmonic is produced using a Quanta Ray Model HG-2-C harmonic generator. Q-switched pulse mode was chosen for its increased fluence compared to long pulse. The modulation frequency, pulse duration, and beam width were dictated by the manufacturer's default specifications for the laser as the controls available to vary these settings did not offer discrete, easily measurable adjustments. Knowledge of these parameters was important due to the dependence of the PA signal upon such things as irradiation duration and modulation frequency [22], and the default settings were the only reliable way to know these parameters. The modulation frequency was fixed at 10 Hz, and the pulse duration was fixed at 8 ns per pulse. The default beam diameter of 8 mm was used initially; though it was later physically trimmed to 4 mm (see Section 2.3.1).

#### Section 2.2.2 Detector

As the photoacoustic signal is sound, a natural choice for a detector is a microphone; however it can be difficult to separate environmental noise from the PA signal with this type of detector. Presumably, the high PA signal produced with the Nd:YAG light source would help in achieving a satisfactory signal-to-noise ratio, as the PA signal is proportional to incident light intensity. However, the q-switching and cooling pumps required to operate the Nd:YAG increased the background noise too much to permit the microphone to be used. A lock-in amplifier can be used to selectively record the signal, but early tests with this system showed that our lock-in amplifier (Stanford Research Systems SR850) did not sufficiently remove environmental noise to achieve a satisfactory signal-to-noise ratio.

Most of these problems were overcome through the use of a piezoelectric detector (Knowles BU-21771, Figure 2-4 for pictures). Because the coupling of vibration between gasses (air) and solids is poor, acoustic noise in the laboratory was eliminated by the use of a piezoelectric. As an added benefit, the detector proved physically more robust than previous microphone detectors. A Teflon block was milled to secure the piezoelectric device, with a hole for the cable leading off of the piezoelectric device running along the interior length of the block (see Figure 2-3 for a schematic). Clear nail polish (an insulating, easily applied adhesive) and electrical tape were both used to secure the piezoelectric in place (see Figure 2-2a and 2-2b, above). These materials were chosen due to the ease with which they could be removed to repair the piezoelectric device in the event that a BNC wire disconnected from the detector. The end result of these efforts was a sensitive and robust detector.



Figure 2-3: Schematic of the Teflon block used to mount the piezoelectric detector. The views from (a) the detector side, (b) opposite the detector side, (c) perpendicular to detector side, and (d) side where the microphone jack was protruding from the center of the block are shown with dotted lines to indicate internal features not visible from the view angle.



Figure 2-4: Pictures of the Teflon piezoelectric mount from the (a) detector surface, (b) microphone/BNC cable jack, and (c) side ends of the Teflon block.

The PA signal strength is inversely proportional to the distance traveled by the signal from origination point to detector. [23] The detector was aligned on the cuvette at the same height position as the laser beam in order for the signal to travel the shortest distance from the generation point to the detector. This also allowed for a consistent reference point for where to aim the laser beam on the sample holder.

Section 2.2.3 Sample holder

The sample holder (also referred to as the sample "cell") had to be optically transparent to 532 nm light, able to withstand the high fluences encountered with a Qswitched laser, capable of coupling to the detector, and easily removed in order to clean or change it to handle different types of samples. A quartz (and later glass) 1 cm pathlength cuvette manufactured for use in visible spectroscopy measurements was chosen due to its meeting all of the above criteria. The cuvette chosen had a cap that was used to seal the cuvette for some initial





measurements. Coupling of the cuvette to the piezoelectric detector was accomplished by clamping the piezoelectric in contact with the cuvette. Silicone-based vacuum grease was used to reduce mechanical noise that may have been associated with vibrations in the room carrying through the cuvette to the detector (the optical table used was not vibrationally isolated). The grease was not found to lubricate or cushion the connection so much that the PA signal was masked, so its use was continued. Typically, a small drop of grease was sufficient to couple the cuvette to the detector. Figure 2-5 presents a schematic layout of the sample cell mount from the side opposite the laser (Fig. 2-5a) and the top (Fig. 2-5b). Pictures of the actual holder from the same views are presented in Figure 2-6.



Figure **2-6**: (a) Side and (b) top view of sample holder. a - female microphone connector for detector, b - Teflon block, c - cuvette, d - back plate of clamp, e - irises for beam alignment (background), f - clamp mounting post, g - clamp arm, h - laser beam path.

#### Section 2.2.4 Signal Processor and the Signal

There were three components to the signal processing equipment: a photodiode, a preamplifier, and a digital phosphor oscilloscope (Tektronix DPO 4104B Digital Phosphor Oscilloscope, 1 GHz, 5 GS/s [GS/s = giga samples/second]). As the oscilloscope was capable of measuring voltage from BNC inputs, the photodiode was wired such that light shining on the diode would produce a voltage proportional to the intensity of the light impinging upon it. Because this arose from the backscattered laser, this was used to trigger data collection. The signal from the piezoelectric device was sent through a preamplifier prior to it being received by the oscilloscope. The oscilloscope itself was chosen as the measurement method due to its ability to resolve sub-nanosecond features of a waveform, the wide range of signal voltages it could process, and its built-in signal processing capabilities. Like many of the components of the system, an oscilloscope is readily adapted to different instrumental schemes and sample types. Both the photodiode (Figure **2-2a**) and the preamplifier were wired by the electronics shop of the Penn State University Chemistry Department.

Oscilloscope measurements require a trigger to begin signal collection. Ordinarily in PA measurements the light modulation device is used to trigger the signal collection device directly, however the built-in Q-switching pulse mode for the Nd:YAG did not have a digital output with which to trigger the oscilloscope. The photodiode fulfilled this function such that each laser pulse was the trigger used to define  $t_0$  on the oscilloscope. The laser beam itself was aimed directly at the sample cell, however the photodiode was able to detect backscattered photons while positioned adjacent to the beam. The photodiode was wired backwards to an 18 V power supply (comprised of two 9 V batteries in series) such that photons hitting the diode surface would cause a voltage to be outputted to a BNC cable connection. The cable linking the photodiode to the oscilloscope was terminated with a 50 Ohm terminal to reduce line noise (Figure 2-2d). When the voltage of the photodiode rose above 2-3 V, the trigger threshold was crossed and measurement began.

When using pulsed light sources, the duration of the thermal wave is longer than the duration of both the light pulse and the acoustic generation process. [24] The time elapsed between acoustic generation and acoustic detection depends upon the thermal transfer efficiency between the sample and the detector, with interfaces between two different types of materials increasing the time. The elapsed time between irradiation and signal detection has been reported as short as a few microseconds [25]; however we have found that our system requires approximately 0.10 ms for the same (see Figures 2-7 and 2-8). PA signal propagation time appeared to be independent of laser energy or analyte concentration (see Chapters 4 and 5 for an evaluation of these results).

The PA signal manifested itself as a single peak with a steep rise and slightly shallower decline (Figure 2-8). It was observed that control solutions containing no analyte had a similar peak shape; however the magnitude of such a peak was several orders of magnitude less intense than the peak observed with analyte present (Figure 2-7). After each PA signal peak, the baseline was observed to have several small pseudo-sinusoidal fluctuations ascribed to either background noise or resonating vibrations in the cuvette. As these fluctuations were not observed by the oscilloscope on the piezoelectric detector channel in between  $t_0$  and the appearance of the PA peak, it seems safe to assign them to cuvette resonance. These fluctuations were observed in sample blanks at an

intensity similar to the initial PA peak itself, which lends credence to this assignment. Other laser PA studies [26, 27] have shown similar peak features even though the timescale between irradiation and PA signal detection varies depending on the system being used to collect the data. [28] The solvent, the sample holder, the distance between the detector and the analyte, the number of interfaces the signal must cross, and the depth within the analyte from which the signal originates all affect the length of time it takes for the detector to pick up on the PA signal. [23]



Figure 2-7: Background signal typical of pure toluene. Even under high laser energy levels (18 mJ/pulse) the signal was rather weak.



Figure **2-8**: Photoacoustic signal produced by gold nanoparticles in toluene (0.0114% (m/m) AuNP/toluene). Laser energy was set to 18 mJ/pulse at 532 nm.

#### Section 2.2.5 Assembly of the instrument

The laser was operated using the control module (see Figure 2-2c) and separately from the rest of the equipment as it had no readily available digital control connectivity. Prior to collecting data, a laser energy meter (ThorLabs PM100D compact power and energy meter console equipped with a ThorLabs ES220C pyroelectric energy sensor) was used to record the average laser energy and standard deviation in milliJoules. The average and standard deviation were calculated using a minimum of 1,000 laser pulses in all tests due to the instability of the laser at some of the chosen energy levels. Standard deviations of less than 10% of the laser energy were typically obtained.

The beam was directed through two irises mounted along the same optical rail post so as to ensure that the beam would be a constant diameter across with limited source-originated scattering, and irradiation of the sample cell cuvette at the same location with each sample. The photodiode was positioned such that its face was orthogonal to the beam direction, placed just on the edge of the beam. This allowed the photodiode to be triggered without blocking the beam or requiring the use of beamsplitters, which frequently were optically damaged by the use of the Nd:YAG, even when Nd:YAG specific components were utilized. A BNC cable connected the photodiode to Channel 1 of the oscilloscope so as to trigger the oscilloscope to collect a measurement every time the photodiode was activated by the laser pulse.

Channel 4 of the oscilloscope was connected to the piezoelectric device through the preamplifier set between 2,000x gain and 2x gain. For every sample the gain was adjusted down so as to avoid saturating the output capabilities of the preamplifier while also maximizing the signal received by the oscilloscope. The preamplifier was connected with a microphone-to-BNC cable to the piezoelectric device, which in turn was connected to the sample cell by way of a clamp pressing the cuvette against the flat, active surface of the device.

#### Section 2.3 Considerations to Improve Measurement Reliability

#### Section 2.3.1 Specifics of Laser Beam Handling

The beam was consistently aimed at the center of the 1 cm wide cuvette so as to not illuminate the piezoelectric device directly with the edges of the beam; it was found that non-focused light was still able to irradiate the piezoelectric due to the width of the beam, which was nearly as wide as the cuvette itself. Due to the aluminum finish on the detector only being 91% reflective of 532 nm light [29], photons that fell on the piezoelectric surface directly were found to increase background noise through absorption. Therefore irises were employed to physically narrow light the beam width to 4 mm. This restriction also aided in aligning the cuvette in the beam.

An additional set of measures taken to improve the consistency of instrument characteristics from sample-to-sample was to leave the cap off for measurements. This still produced a measureable PA signal while preventing the disturbance often caused by removing and replacing the cap. Furthermore, the cuvette was left in place while rinsing in between samples so as to keep the beam alignment and coupling to the piezoelectric device consistent.

The Nd:YAG was capable of outputting 200 mJ/pulse at 532 nm, however it was found that only a fraction of this output could be used for several reasons. The cuvettes used as sample holders were incapable of withstanding much more than 50 mJ/pulse

without experiencing damage to the transparent faces. Additionally, the AuNPs would adhere to the cuvette surface above a certain absorbance level, which was found to depend on laser energy and nanoparticle concentrations. This limited the usable laser energy to 21 mJ/pulse or less.

#### Section 2.3.2 Signal Processing Specifics

The preamplifier used was capable of providing discrete gain factors of 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000. As the NP concentration or laser energy were increased, the gain was adjusted down to accommodate the more intense signals due to limits on the output voltage of the preamplifier. Data processing accounted for these differences in gain across different experimental parameters (e.g. laser energy and analyte concentration) by dividing the signal received by the oscilloscope by the gain factor used to amplify it. In this way, the full range of the oscilloscope could be better utilized so as to minimize the percent error of the measurements and avoid signal saturation. The maximum gain used was 2000x, and it was for the blank control solutions.

It is common in radiofrequency measurements to terminate the end of a cable carrying a signal so that the signal will not be reflected back into the cable and interfere with the incoming signal. In this case the BNC cables connected to the oscilloscope were connected to T-junctions with 50 Ohm terminators. The oscilloscope parameters themselves were chosen such that a single laser pulse would be recorded with each oscilloscope measurement, thereby also measuring a single photoacoustic waveform. A window of 1 ms (100  $\mu$ s/div over 10 divisions) provided this width of measurement. The background noise with a single oscilloscope measurement was high relative to the signal

strength, so signals were analyzed by the oscilloscope by averaging 64 measurements together and displaying the result on the screen. The next lower available signal count number (32) still left an undesirable amount of noise in the signal, however the next higher available signal count number (128) did not offer a noticeable improvement given the extra time required for data collection.

#### Section 2.4 Conclusions and future directions

A photoacoustic instrument was successfully assembled and constructed from both existing and in-house designed and constructed components. In constructing the instrument it was found that open cell measurements were not only possible, but also preferred in order to make sample loading more consistent. Oscilloscope measurements had to be performed over an average of 64 measurements to adequately reduce noise in a timely fashion where each measurement was triggered off of the laser pulse *via* photodiode. Microphone detectors were too sensitive to environmental acoustic noise to be used for this technique. Instead, piezoelectric detectors were used as they were adequately selective for the PA signal.

A wider variety of NP shapes, sizes, and compositions may provide a better understanding of the performance of the instrument in terms of PA peak detection time, waveform shape, or signal intensity. Furthermore, adjustments to the instrument itself, including more secure mounting arrangements for the optical handling equipment, may result in more consistent measurements. In order to more thoroughly explore abnormalities in signal shape encountered at higher laser energy levels and NP concentrations, an alternative PA measurement, known as a photothermal lensing technique, could be explored. This would be useful in observing any microscale boiling of the solvent. This is discussed in more detail in Chapter 7.

### Chapter 3

## **Equations Governing the Photoacoustic Signal**

#### Section 3.1 Equations Governing the Photoacoustic Signal

The photoacoustic (PA) signal is a measure of the sound wave pressure produced by the analyte of interest. The thickness of the absorbing material, its absorption coefficient at the excitation wavelength, and the modulation frequency of the light source impact the signal strength itself. However, only a certain percentage of the acoustic pressure is transduced to an electric signal by the detector. This percentage depends significantly upon the geometry and thermal properties of the cuvette and the detector, the arrangement and type of the detector, and the number of thresholds the acoustic signal must cross *en route* to the detector (i.e. solvent to cuvette and cuvette to detector). The percentage of the acoustic signal that is picked up by the detector must be determined if the instrument is to be used to supplement studies which use the NP heat to drive chemical reactions by quantitatively determining the absorption coefficient of a variety of analytes and the magnitude of the heat released.

Equation 3-1 shows the proportional relationship between photoacoustic amplitude and the key variables characteristic of the instrument and the analyte. [23] This proportional relationship applies to "cylindrical" acoustic sources like the one used in this work, which are characterized by optical penetration depths into the absorbing media that are greater than the radius of the light beam itself. The proportionality in Equation 3-1, which relates photoacoustic pressure (P<sub>A</sub>) to laser energy (E), volumetric expansion coefficient of the solvent ( $\beta$ ), absorption coefficient of the analyte ( $\epsilon$ , in (m/m)%<sup>-1</sup> concentration units), speed of sound in the solvent (v), heat capacity of the solvent ( $C_p$ ), distance from the acoustic source to the detector (r), and radius of the laser beam (R), can be treated as an equality if an instrument constant, *k*, is determined (Equation 3-2).

$$P_A \propto \frac{E\beta \alpha v^2}{C_p \sqrt{r} R^{3/2}}$$
 3-1

$$P_A = \frac{kE\beta\alpha v^2}{C_p \sqrt{rR^{3/2}}}$$
 3-2

k is a measure of how well energy is propagated from the NPs to the detector through the various boundary layers in between. It is known that thermal energy such as sound waves can be reflected quite well at such interfaces [22, 30], so k is expected to be less than 1— indicating that the PA pressure detected is less than the PA pressure produced by the NPs.

Dimensional analysis of the units associated with this proportionality reveals units of amount over distance and time squared. Conversion of these units into their SI counterparts reveals the constants to equal pressure in Pascals (Pa):

$$P_A \propto \frac{mol}{cm \cdot s^2} \to \frac{kg}{m \cdot s^2} = Pa$$
 3-3

As these constants produce units of pressure, the correction constant, k, which is required to equate the instrument signal with the constants aforementioned, will be a dimensionless number.

There is reasonable concern that the instrument constant obtained by Equation 3-2 will not be the same from analyte to analyte because the instrument was disassembled and reassembled in between studying the two primary analytes examined: gold and iron oxide (magnetite) nanoparticles (NPs). Furthermore, disassembly of the instrument was required between some measurements of the same analyte. Due to the nature of the instrument assembly, the transfer of thermoacoustic energy from the NPs to the solution

to the cuvette through the vacuum grease and to the piezoelectric may change in efficiency from assembly to assembly. It is surmised that if k is significantly different between the two analytes that the instrument's capabilities will be severely limited without further modifications.

#### Section 3.2 Instrument Constant Calculated from the Gold and Iron Oxide NP Results

The instrument constant, k, will be calculated from the slope of data found with both analytes in this work (Chapters 4 and 5). It is worth considering here how this will be done. The PA signal is treated as a linear equality as follows, where the x-variable, c, is the concentration of the analyte in solution in (mass/mass) % ratio with the solvent.

$$y = mx + b 3-4$$

$$P_A = \frac{kE\beta\varepsilon v^2}{C_p\sqrt{rR^{3/2}}}c + 0$$
3-5

$$m = \frac{kE\beta\varepsilon v^2}{C_p\sqrt{r}R^{3/2}} \to k = \frac{mC_p\sqrt{r}R^{3/2}}{E\beta\varepsilon v^2}$$
3-6

Piezoelectric devices tend to work best as signal detectors when direct mechanical coupling between the signal transfer medium and the detector is possible, such as is found with solid and liquid samples. [31] This is to say that signals either originating from or traveling through gasses do not get efficiently transferred to a piezoelectric device. This happens to make the piezoelectric device ideal for detection in acoustically noisy environments, such as those encountered while using our Nd:YAG laser. Furthermore, piezoelectrics are useful with pulsed light sources due to their fast response times [31].

The ceramic material contained within piezoelectric devices has a characteristic constant known as the voltage sensitivity  $(S_v)$  which determines the voltage output
provided per acceleration (g). In the case of the Knowles Electronics Model BU-21771 piezo-ceramic accelerometer used in this work, the  $S_v$  was provided in decibels relative to 1.0 Volt/g, and at the modulation frequency used by the Nd:YAG laser, 10 Hz,  $S_v$  was -45dB. [32] Using

$$L_{dB} = 10 \cdot \log_{10} \left(\frac{P_1}{P_0}\right) \tag{3-7}$$

where  $P_1/P_0$  is the ratio of the piezo-ceramic voltage sensitivity in Volt/g to 1.0 Volt/g.  $S_v$  of -45dB was equivalent to  $3.1623 \times 10^{-5} \text{ V} \cdot \text{g}^{-1}$  and could be used to convert measured Volts to acceleration. As the photoacoustic equations used were in photoacoustic signal units of Pascals, the mass and contact surface area of the piezoelectric detector were used to convert the acceleration experienced into pressure in Pascals as follows:

$$P_A = \frac{m \cdot g}{l \cdot w} \tag{3-8}$$

where *m* is the mass of the detector in kilograms, *g* is the acceleration calculated from the voltage sensitivity in m·s<sup>-2</sup>, and *l* and *w* are the length and width of the contact side of the piezoelectric detector, respectively, in meters. Rearranging 3-7 to solve for the voltage sensitivity results in 3-9, the result of which can be used in the calculation of a constant that is applied to the piezo-ceramic voltage to obtain pressure (3-10)

$$P_1 = 10^{\left(\frac{L_{dB} \cdot P_0}{10}\right)}$$
 3-9

$$Pressure = 2.030815 x 10^5 \frac{kg}{m \cdot s^2 \cdot V} \cdot L_{dB} \qquad 3-10$$

where  $L_{dB}$  is the voltage level given off by the piezoelectric.

The variables for heat capacity and absorption coefficient must be converted into SI units to achieve Pascals, however it is helpful for experimental purposes to further alter the absorption coefficient by separation into the molar absorption coefficient and the concentration as follows:

$$\alpha = \varepsilon c$$
 3-11

Separating the absorption coefficient into the molar absorption coefficient and the concentration allows for facile determination of the absorption coefficient with UV-Vis spectroscopy. While PA analysis is routinely used to determine the absorption coefficient of an analyte of known concentration, all photoacoustic instruments have correcting constants associated with them that cannot be determined without prior knowledge of the absorption coefficient as obtained by other means. Therefore separation of these two terms is beneficial, as the concentration domain will often be used in determining the response of the instrument. The nanoparticle batches used in these measurements were not monodisperse, so molarity was an unreliable measure of the concentration. Therefore, the mass percent ratio of metal nanoparticles to solvent was used as the concentration units.

The absorption coefficient of a nanoparticle sample, when known in conjunction with the dimensions of an irradiated volume and the irradiation energy, can be determined using pulsed photoacoustic spectroscopy. [33] Characterizing the instrument constant requires knowledge of the absorption coefficient of the analyte, though. UV-Vis spectroscopy can reveal the extinction coefficient of an analyte, however the scattering portion of the extinction coefficient does contribute to the PA signal. Mie theory for the scattering of light by particles smaller than the wavelength of light in question can be used to determine what the scattering coefficient is for the nanoparticles used. True analytical calculations with Mie theory are computationally intense, however the quasistatic approximation can be used to calculate the scattering coefficient if the dielectric constants for the system and the nanoparticle sizes are known. The following approximation is used to find the dimensionless scattering coefficient,  $Q_{sct}$ . [34]

$$Q_{sct} \cong \frac{8\sigma^4 \cdot R^4}{3} \left(\frac{\varepsilon_m - \varepsilon_d}{\varepsilon_m + 2\varepsilon_d}\right)^2$$
 3-12

Here,  $\sigma$  is the wavenumber of the light source and R is the radius of the nanoparticle spheres. The dielectric constants  $\varepsilon$  for the metal nanoparticle material ( $\varepsilon_m$ ) and the surrounding medium (toluene,  $\varepsilon_d$ ) are dimensionless as they are relative to the permittivity of a vacuum. Ultimately, it was determined that the scattering coefficient was a negligible contributor to the extinction coefficient, so the absorption coefficient was, within error, equal to the UV-Vis determined extinction coefficient.

With this information, the scattering coefficient can be subtracted from the extinction coefficient determined experimentally using UV-Vis absorption of the nanoparticle solutions to obtain the absorption coefficient. The ratio of this value to the value obtained by the PA instrument is the instrument constant. What remains, then, is to determine  $\varepsilon_{Abs}$  from UV-Vis and use it with the photoacoustic signals for our NP systems. I describe this process in the next two chapters.

# Chapter 4

# Measuring the Photothermal Response of Gold Nanoparticles

### Section 4.1 Introduction

Gold nanoparticles (AuNPs) served as the first analyte for this system, and were used to explore the limits of excitation energy, optical density, and duration of measurements made by the instrument. AuNPs are known to absorb well at 532 nm [35], and TEM and UV-Vis data was collected to support the notion that the 532 nm absorption by the nanoparticles was largely stable under the conditions used. Equations governing the PA signal (Chapter 3) were used to evaluate the data to attempt to quantify the pressure and heat given off by the AuNPs as measured by the system.

### Section 4.2: Analyte and Photoacoustic Measurement Parameters

The AuNPs used were made using the Brust method [36] with dodecanethiol ligands and had an overall average diameter of  $2.05 \pm 0.51$ . Using a 1:10 (v/v) mixture of dodecanethiol and toluene as a solvent, AuNP solutions were made at concentrations ranging from 0.00% to 0.0114% (m/m ratio of AuNP to toluene) using a single stock solution of 0.114% AuNP:toluene for sample preparation.

The PA instrument utilized 10 Hz, 8 ns laser pulses at 532 nm with energies from 3 to 21 mJ/pulse. The beam was reduced to 4 mm diameter using a series of irises. The pre-amplifier connecting the piezoelectric detector to the oscilloscope was adjusted from 2000x gain (for blank toluene solutions) to as low as 2x gain as laser energy and concentration increased (up to 0.0114%). Gain was adjusted to prevent saturation of the

signal peak as described in Chapter 2. The oscilloscope was set to collect the average of 64 individual measurements, each one of which contained one photoacoustic waveform (corresponding to one laser pulse). The oscilloscope was triggered to collect these measurements using a photodiode arranged to detect the laser pulse, which lasted and dissipated several orders of magnitude faster than the photoacoustic signal. Data collected was normalized to eliminate the background signal by subtracting the average waveform of 10 measurements (each of 64 pulses) at each laser energy level for the blank toluene solution from the average waveform collected from ten 64 pulse measurements of the other solution concentrations at the corresponding laser energy.

#### Section 4.3: Characterization of Nanoparticles

#### Section 4.3.1: Transmission Electron Microscopy

Transmission electron microscopy (TEM) images of the AuNPs used showed them to be relatively monodisperse with an overall average diameter of  $2.05 \pm 0.51$  nm (Figure 4-1). Measurements were made using ImageJ software with 100 NPs from each image measured. The spherical particles upon irradiation experienced minor but noticeable size changes (3 mJ laser pulses for 60 minutes). Histograms are overlaid with the corresponding AuNP samples in Figure 4-1, and it can be seen that given such a relatively high standard deviation, the size change was not significant. A similar effect of irradiation is observed with an even more concentrated solution, 0.0285%. The AuNPs went from 2.15  $\pm$  0.52 nm to 1.88  $\pm$  0.46 nm. These slight changes in size did not accompany a change in shape, though, as spherical particles were observed both before and after laser irradiation.



Figure **4-1**: Gold nanoparticles. a: 0.0114% gold to toluene (m/m),  $1.94\pm0.43$  nm. b: 0.0114% gold to toluene (m/m), after irradiation by 3 mJ/pulse laser light for 60 minutes,  $2.27\pm0.53$  nm. C: 0.0285% gold to toluene (m/m),  $2.15\pm0.52$  nm. d: 0.0285% gold to toluene (m/m) after 3 mJ/pulse laser light irradiation,  $1.88\pm0.46$  nm. TEMs collected February 20, 2013 by Robert Johnson.

## Section 4.3.2: Electronic Absorption Spectra

The slight size changes observed in the TEMs made it necessary to examine the UV-Vis spectra to reveal any change in absorptivity at 532 nm, as this would affect our measurements over time. The UV-Vis spectra showed a small change in the AuNP solutions following irradiation (Figure **4-2a** and **4-2b**) where irradiation caused a small

increase in the plasmonic feature around 500 nm. These changes were not, however, accompanied by a significant change in absorptivity at 532 nm. Absorbance at 532 nm was linearly dependent upon AuNP concentration in toluene both before and after irradiation (Figure **4-2c**), suggesting that PA signals should also be linearly dependent upon concentration both before and after irradiation, since the PA signal is proportional to absorbance. [23]

As these nanoparticles were not sharply monodisperse, the extinction coefficient units utilized in the photoacoustic calculations were in terms of (mass/mass) % (AuNP/toluene), as molar concentrations would require uniform particle diameter. In the case of these AuNPs, the extinction coefficient before irradiation was 5954.4 (m/m%)<sup>-1</sup> and 5954.5 (m/m%)<sup>-1</sup> after, so practically no change was observed.

The importance of this lies in the duration of each experiment. If the nanoparticles experienced significant optical density changes at the excitation wavelength as excitation duration increased, the experiments performed with each AuNP sample would be limited in number and duration. The ability to disregard these time constraints enabled experiments to be carried out over long periods of time. For the data in Figure **4-2c**, the most concentrated solutions measured *via* UV-Vis were irradiated with laser light, however they were too optically dense to be reliably measured with the UV-Vis instrument. Therefore, following irradiation, they were diluted in order to stay above 10% transmittance (below 1 a.u.), and the absorption value at 532 nm was subsequently multiplied by the dilution factor.

AuNP concentration (m/m% of gold to toluene)	Absorbance at 532 nm prior to laser irradiation	Absorbance at 532 nm after irradiation at 3 mJ/pulse
0.0011%	0.0491	0.0509
0.0023%	0.1087	0.1092
0.0057%	0.2794	0.2542
0.0086%	0.4811	0.5026

Table 4-1: Absorption at 532 nm by AuNP before and after laser irradiation.



Figure 4-2: (a.) AuNP UV-Vis absorbance spectra prior to laser irradiation. (b.) UV-Vis spectra following irradiation for 60 minutes by 3 mJ/pulse laser light at 10 Hz, where the spikes in absorbance at 490 nm and 660 nm, along with a small bump at 580 nm, are no longer seen. (c.) Absorbance at 532 nm for AuNPs before and after laser irradiation. Nanoparticles were dissolved in a solution that was 1:10 (v/v) dodecanethiol to toluene. Irradiation times represent the length of time the laser was on firing pulses at 10 Hz. After 5 min. this equated to 3,000 pulses (24  $\mu$ s total exposure); after 60 min. this equaled 60,000 pulses (480  $\mu$ s total exposure). Absorbance values above 1 a.u. were extrapolated from diluted samples of the more concentrated solutions that were actually irradiated.

#### Section 4.4: Dependence of Photoacoustic Pressure on Irradiation Time

The minimal perturbation of AuNP size and absorptivity upon irradiation allowed the pursuit of PA studies. The first study was to confirm that the PA signal did not change with laser irradiation time, as a lack of change in the UV-Vis spectra for this variable would indicate a change should not be observed. Figure **4-3** confirms that a constant photoacoustic signal is produced by 3 mJ/pulse pulses as a function of time. However, greater fluctuations were observed for the higher concentrations of NPs which gave off proportionally higher PA pressures. The fluctuations were within the standard deviation of the measurements (see Table **4-2**). The proportional relationship between PA pressure and NP concentration matches the relationship between absorbance and concentration absorbed in the UV-Vis spectra. This result, coupled with the small changes observed *via* TEM and UV-Vis spectroscopy showed that irradiation time should not appreciably impact the photoacoustic signal.

An interesting observation with the consistency of the PA pressure measured in Figure 4-3 is the fluctuation in signal strength as laser irradiation time goes on. The fluctuation is significant at higher NP concentration; however the pressure itself is also larger for those solutions. Table 4-2 displays the PA pressure average for each concentration, calculated by averaging each data point from time 0 to time 60 minutes. The standard deviation displayed is the standard deviation of that average, and it is observed that the standard deviation increases as PA pressure increases. Figure 4-4 plots this data as standard deviation versus PA pressure average, showing that there is a reasonably linear ( $R^2 = 0.966$ ) relationship between standard deviation and pressure.



Figure 4-3: AuNP PA signal dependence on laser irradiation time. Legend entries are in units of mass percent ratio of AuNPs to toluene. Irradiated with  $3 \pm 0.2$  mJ/pulse laser light at 4 mm beam diameter and 8 ns pulse width. Standard deviation of each point from the average across all points in each set is presented with vertical bars.

0	0 1
AuNP	Average PA Signal from 0 – 60 minutes
Concentration	irradiation time, with Standard Deviation.
0.0000%	2.21 ± 1.09 Pa
0.0011%	80.41 ± 3.90 Pa
0.0023%	545.4 ± 11.8 Pa
0.0057%	1930 ± 79 Pa
0.0086%	3972 ± 124 Pa
0.0110%	4904 ± 227 Pa
0.0285%	8529 ± 303 Pa

Table 4-2: Average PA Signal over 60 minutes of irradiation at 3 mJ/pulse.



Figure 4-4: Dependence of the standard deviation of the PA pressure on the magnitude of the detected PA pressure. With a linear  $R^2$  of greater than 0.95, the standard deviation shows a linear dependence on PA pressure, suggesting that the apparent variance in the signal for the 0.0285% solution in Figure 4-3 should be expected.

Section 4.5: Dependence of PA Waveform on AuNP Concentration and Laser Energy

The PA waveforms can reveal information as to the stability of the measurement at various optical densities and laser energies. As described previously in Chapter 2, Section 2.4, the waveforms typically have a baseline pressure of 0 Pa leading into a sharp rise culminating in a peak at roughly 100  $\mu$ s following the trigger at time 0 ms. The trailing edge of the waveform is shallower, occasionally marked by bumps and shoulders. The final part of the waveform is a slightly negative pressure experienced as a result of the recoil of the piezoelectric ceramic to the rarefaction component of the pressure wave. Though the magnitude of the peak will be used for most quantitative discussions of the PA signal, the shape of the waveform is still worth discussing. The control solution with no AuNPs showed an increase in signal features as laser energy increased, but the peak PA pressure detected with the control was still several orders of magnitude less than the intensity of the pressure detected with AuNP-containing solutions. Nonetheless, the waveform produced by the control was subtracted from the waveforms produced by other solutions at corresponding laser energy levels to normalize them to the background of the instrument.

Figure 4-5 displays the changes in signal shape typically encountered as the AuNP concentration increased at each laser energy value. The shape of the rising portion of the signal was uniform across the range of concentrations measured and the range of laser energy levels utilized. The changes in waveform shape were most pronounced on the declining side of the signal waveform (tailing edge). These changes routinely manifested as small recurring peaks emerging out of the trailing edge and were not

observed to be significantly dependent on concentration after 0.40 - 0.50 ms following the laser pulse trigger.

Within the sample cell, the PA signal propagates outward from the laser beam path in all directions. [22] The initial PA signal detected is the PA signal traveling directly towards the detector from the irradiated solution. The PA signal propagating outward in other directions, upon encountering boundaries between materials, such as between the solution and the cuvette and the cuvette and the piezoelectric detector, will be at least partially reflected back into the solution. The pressure fluctuations observed in the waveform following the peak are attributed to these reflected signals. The PA signal strength decreases with the square root of the distance from the origination point, so these reflected signals would be very weak upon detection. [23] In more concentrated samples, or in samples with stronger laser energy levels, these reflected signals were likely strong enough to be greater than the tailing portion of the initial PA peak and emerge as the observed bumps and shoulders after the peak of the signal.

An additional point of interest is shown in Figure **4-5a**. Here, the signal produced by the original stock solution of AuNPs in dodecanethiol:toluene is presented with the PA signals produced by less concentrated solutions irradiated by the same laser energy. The stock solution had an optical density such that the laser beam was attenuated at a depth roughly equal to the diameter of the laser beam itself, thus the laser failed to irradiate the full pathlength of the cuvette. This PA signal generation method is known as spherical source generation. [23] The change in generation point is assigned as the cause of the drastic decrease in PA signal in the 0.1140% solution, as well as the cause of the shallow decrease in the PA signal following the peak. The PA signal is propagating



Figure 4-5 PA signal shape dependence upon AuNP concentration. Laser energy increases from a.-f. in the order 3, 8, 12, 15, 18, 21 mJ/pulse (each with a standard deviation of 0.2, 0.7, 0.5, 0.6, and 0.7 mJ/pulse, respectively). Each waveform is the average of ten measurements made at each concentration and laser energy combination. Legend in a. is valid for b.-f.

radially outward from the cuvette surface, as opposed to from the cylindrical beam path, potentially promoting constructive and destructive patterns of pressure waves that differ from those displayed by the lower concentration solutions. In total, this work shows that it is the peak signal that provides the least ambiguous interpretation, and we use that in the future.

Upon observing the limits to the system with regard to the PA pressure strength, a few AuNP solutions were focused upon to measure the dependence of the system on laser energy. If the full PA waveforms are overlaid at the same concentration but with changing laser energy levels, it can be further observed that the initial rise of the PA signal does not vary in shape or peak detection time across increasing laser energy, however the tailing edges of the PA signal contain more shoulders and small peaks as the laser energy increases (Figure 4-6). In the case of the control solution, comprised of dodecanethiol and toluene, a PA signal of the same general shape as the AuNP signal is observed at higher laser energy levels. The intensity of this peak is roughly 3 orders of magnitude less intense than the PA signals of AuNP-containing solutions at corresponding laser energy, suggesting that the background signal of the system is of little significance to the sample signals. Waveforms were normalized here as they were for Figure 4-5. The solvent for all measurements was comprised of a 1:10 (v/v) mixture of dodecanethiol in toluene in an effort to prevent NP aggregation via the photolysisinduced loss of the stabilizing ligands on the NPs. This decrease accompanies a broadening of the tailing edge of the waveforms (Fig. 4-6), and it can be seen in Figure 4-**6c** that several waveforms peak at the same PA pressure even with different excitation energy. It is also observed that the waveforms broaden in the tailing edge. While it is

presently unknown, the mechanism responsible for the broadening may be responsible for the lack of increase in PA pressure as energy increase.



Figure **4-6**: PA waveforms' dependence on laser energy across constant AuNP concentration. From a.-c. the m/m% (AuNPs to toluene) is 0.0000%, 0.0057%, and 0.0114%. Each waveform is the average of ten measurements made at each concentration and laser energy combination. The waveforms in a. are not normalized *via* subtraction of the blank toluene solution signal, as these are the blank toluene solution signals and are flat lines at 0 Pa following normalizing. Waveforms in b. and c., however, are normalized by subtracting the blank toluene waveforms obtained at the same laser energy levels. Standard deviation of laser energy measurements are 0.2, 0.7, 0.5, 0.6, and 0.7 mJ/pulse for 3, 8, 12, 15, 18, and 21 mJ/pulse, respectively.

Section 4.6: Linear Response of Peak PA Pressure to AuNP and Laser Energy

It then is of interest to determine if the peak increases linearly with concentration as should happen according to the PA pressure equations. [23] The extinction coefficient showed a linear increase in absorbance at 532 nm with UV-Vis spectroscopy, and the PA signal did linearly increase with increasing AuNP concentration, as well (Figure 4-7). At high laser energy the signal response began to level off at high AuNP concentration. It is likely that this is due to a saturation of the photothermal transfer efficiency of the system to the detector at such energy levels. Linear fits were still reasonably good with R<sup>2</sup> values above 0.9 (Table 4-4).

The linearity of the PA signal response to laser energy was marginal, with  $R^2$  correlation coefficients well below 0.9 (Figure **4-8**). This deviance was not a simple saturation of the detection capacity of the system, as the peak maxima actually decrease



Figure 4-7: PA pressure dependence upon AuNP concentration. Legend units are mJ/pulse for applied laser energy. At low energy the PA signal increased linearly with concentration, however high laser energy levels produced steep changes in PA signal with concentration followed by a flat response, indicating some kind of saturation of the signal production mechanism. Standard deviation of each point from the average of ten measurements collected at each concentration and laser energy is presented by bars (hidden by data points). Standard deviations of laser energy pulses are 0.2, 0.7, 0.5, 0.6, and 0.7 mJ/pulse for 3, 8, 12, 15, 18, and 21 mJ/pulse, respectively.

at the highest laser energy for both 0.0057% and 0.0114% solutions. As discussed previously (*Section 3.5*), the cause of the tailing edge broadening in the waveforms may be responsible for the lack of linear increase in PA pressure with increasing energy.

Irradiation Energy (mJ/pulse)	Linear Equation	$R^2$
3	$y = (5.2 \pm 1.1)e7x + 37$	0.9995
8	$y = (1.44 \pm 0.12)e8x + 390$	0.9932
12	$y = (6.71 \pm 0.73)e8x + 2,410$	0.9882
15	$y = (6.73 \pm 0.62)e8x + 2,020$	0.9917
18	$y = (9.0 \pm 2.7)e8x + 8,930$	0.9166
21	$y = (6.7 \pm 2.1)e8x + 6,900$	0.9105

Table 4-3: Linear best fit of AuNP PA pressure peaks vs. concentration



Figure **4-8**: PA signal dependence on laser energy for low concentration AuNPs. Standard deviation of each point from the average of ten measurements made at each concentration and laser energy combination is presented, as well. Standard deviations of laser energy pulses are 0.2, 0.7, 0.5, 0.6, and 0.7 mJ/pulse for 3, 8, 12, 15, 18, and 21 mJ/pulse, respectively.

Table 4-4: Linear best fit of AuNP PA pressure peaks vs. energy

Concentration (m/m %)	Linear Best Fit	$\mathbb{R}^2$
0.0057%	$y = 3.96 \pm 0.91 x - 11$	0.8263
0.0114%	$y = 5.1 \pm 1.4 x - 6.5$	0.7667

#### Section 4.7 Calculation of the Instrument Constant

The PA signal exhibited a better linear response to changing AuNP concentration, so the slopes obtained from the plots of pressure vs. concentration in Figure **4-7** are used to determine the instrument constant at each excitation energy. Using equation 3-6 from Chapter 3, where irradiation energy, E, changes with each data set:

$$m = \frac{kE\beta\varepsilon v^2}{C_p\sqrt{rR^{3/2}}} \to k = \frac{mC_p\sqrt{rR^{3/2}}}{E\beta\varepsilon v^2}$$
3-6

the dimensionless instrument constant can be calculated. If the instrument detects the PA signal with the same efficiency at each excitation energy, k should be the same with each energy. The results of this calculation are found in Table 4-5, and it can be seen that k does change with energy, although it is not changing linearly. The average value across all energies is  $0.359 \pm 0.163$ . The peak k is found at 12 mJ/pulse, suggesting that 12 mJ/pulse is where the acoustic signal is most efficiently detected by the instrument.

Laser energy (mJ/pulse):	<i>k</i> (unitless):
3	$0.17 \pm 0.04$
8	$0.178 \pm 0.018$
12	$0.55 \pm 0.07$
15	$0.44 \pm 0.05$
18	0.49±0.15
21	0.32±0.10

Table 4-5: Instrument constant for AuNP with varying excitation energy

It is known that the intense instantaneous power delivered by pulsed light sources can saturate a PA system, so it is possible that the linearity of the PA response will decrease at high energy as a result. [37] Regarding the plot of PA peak pressure versus excitation energy (Figure 4-8), if the pressure is normalized (divided) by k for the corresponding energy levels, the linearity of the plots improves dramatically, as shown in



Figure 4-9. The PA pressure represented in Fig. 4-9 is the pressure produced at the cuvette edge.

Figure 4-9: AuNP PA peak pressure, normalized to k for each laser energy, and plotted versus laser energy. Standard deviations of laser energy pulses are 0.2, 0.7, 0.5, 0.6, and 0.7 mJ/pulse for 3, 8, 12, 15, 18, and 21 mJ/pulse, respectively.

# Section 4.8: Drawbacks to AuNPs

Higher laser energy levels would have been explored using AuNPs, however it was found that the AuNPs were prone to undesired reactions at higher photon fluences. In the case of this work, the undesired reactions were discovered after observing the PA

waveforms changing shape. This shape change resulted in a peak occurring at a later time than expected. Inspection of the cuvette for abnormalities regarding beam alignment or the quality of the cuvette mount revealed that the NPs had deposited on the inner surfaces of the cuvette where the laser beam was irradiating the solution (Figure **4-10**).



Figure **4-10** (at right): AuNPs deposited on the cuvette surface following measurements made at high laser energies. Lower laser energies also resulted in such depositions with more concentrated solutions.

We suggest this was a significant contributor to the aberrations in PA signal response and shape at high laser energy as such adhesion was not observed after measurements produced more typical waveforms (see Figures **4-5** and **4-6**). The AuNPs adhering to the cuvette surface functioned as an optical filter to the rest of the solution—thereby reducing the laser energy experienced by the bulk solution. Furthermore, the geometry of signal origination is important in determining PA signal strength and waveform. When AuNPs adhere to the cuvette surface, the absorbed energy is subsequently released as a thermal wave from the points of adhesion. This explains these changes in the propagation time of the signal to the detector surface relative to PA signals produced purely from the bulk solution. The delay in propagation time experienced by the thermal wave was due to crossing an additional material threshold (from the solid deposited layer to the liquid phase).

The notion of undesired reactions was further supported by the work of Kaitlin Haas of the Lear group. [38] While irradiating similar AuNPs with higher levels of laser energy, it was observed that the nanoparticles would precipitate out of solution and grow in size to 20 nm spheres, (as seen in Figure **4-11**), a behavior known to intensely irradiated AuNPs. [35]



Figure 4-11: AuNPs precipitated out of solution with toluene following irradiation with 100 mJ/pulse 532 nm laser light. NP solutions were irradiated by 7,000 pulses, at 8 ns/pulse, which equated to 56  $\mu$ s of total irradiation time. TEM images showed the NP size to increase from 2nm to 20 nm. [38]

# Section 4.9: Conclusions

Gold nanoparticles served as a good test system for making adjustments to the photoacoustic instrument. The stability exhibited at room temperature both before and after laser irradiation allowed efforts to be focused on instrument adjustments. The PA response was linear with respect to AuNP concentration and laser energy from 0.0000% to 0.0114% AuNP concentration and 3 to 21 mJ/pulse laser energy. Above these ranges the PA response stopped increasing. Presumably this is due to a limit in detection capability of the system; however the peak PA response of various parameter combinations was not always the same magnitude. It is suspected that NPs adhering to the cuvette surface and changes in the location of origin of the PA signal contribute to the inconsistent limit in PA response. These same measurements should be made in the future on different sizes of AuNPs or NPs with different surfactants to determine if these parameters control the PA signal.

# **Chapter 5**

# Measuring the Photothermal Response of Iron Oxide Nanoparticles

### Section 5.1: Introduction

The choice of magnetite nanoparticles was made to serve as a second broadly absorbing analyte that would also absorb at 532 nm. Fe<sub>3</sub>O<sub>4</sub> NPs have the potential to serve in many of the same capacities as AuNPs as sources of heat for both imaging and reactivity purposes, without the expense of gold, and with the added property of magnetism, which may be adapted to targeting modalities. Yet another difference between the Fe<sub>3</sub>O<sub>4</sub> NPs and AuNPs has to do with electronic properties, as Fe<sub>3</sub>O<sub>4</sub> NPs lack the intense plasmonic band found in AuNPs. It has been found that plasmonic resonance promotes heat transfer [2-4], so it was of interest to see if the photoacoustic waveform shape and intensity produced by Fe<sub>3</sub>O<sub>4</sub> solutions of similar optical density as compared to AuNPs would be different.

### Section 5.2: Characterization of Fe<sub>3</sub>O<sub>4</sub> Nanoparticles

### Section 5.2.1 Transmission Electron Microscopy

The Fe<sub>3</sub>O<sub>4</sub> NPs used had an average diameter of  $5.9 \pm 0.7$  nm as measured by TEM (Figure **5-1a**), which was 3 times wider than the AuNPs used previously. TEM images before and after (Figure **5-1b**) irradiation by 100 mJ/pulse (which lasted for 7,000 pulses, or roughly 12 minutes, equivalent to 56 µs of exposure) showed no change in NP shape or size as a result of irradiation. Visual inspection of the NP-containing vials revealed the NPs remained suspended without crashing out of solution or adhering to the cuvette surface. This was yet another befit to using  $Fe_3O_4$  NPs over AuNPs for the purpose of producing photothermal heat.



Figure 5-1: Fe<sub>3</sub>O<sub>4</sub> NPs (in hexanes) showed stability in terms of both size and solubility under high energy laser irradiation. TEM images showed the nanoparticles to be nearly monodisperse (a) before  $(5.9 \pm 0.7 \text{ nm})$  and (b) after  $(6.0 \pm 0.9 \text{ nm})$  irradiation for 7,000 pulses with 100 mJ/pulse 532 nm laser light. At 8 ns/pulse, this was equivalent to 56 µs of exposure time. The scale bar in the TEM images in (a) and (b) is 20 nm.

### Section 5.2.2 Electronic Absorption Spectra

These were synthesized [39] with oleylamine ligands and were dissolved in hexanes by a colleague (Johnson), but later diluted with toluene to give concentrations up to 0.040% (m/m) Fe<sub>3</sub>O<sub>4</sub>/toluene. The PA pressure intensity should yet again be linearly proportional to absorptivity, so UV-Vis spectra of the target solutions were collected both before and after 3 mJ/pulse laser irradiation to determine if any spectral changes were induced by the laser (Figure **5-2**). There was no observable change in band-shape after irradiation.

Plotting the absorbance at 532 nm against concentration revealed a change in extinction coefficient following irradiation (Figure 5-3)—a result distinct from that found with AuNPs. Table 5-1 shows the absorbance values at 532 nm and the accompanying standard deviations (as determined by the UV-Vis instrument software) for each solution



Figure 5-2: a:UV-vis spectra of iron oxide nanoparticles after 5 minutes irradiation with 3 mJ/pulse laser light. b: UV-Vis spectra of iron oxide NPs following prolonged  $3 \pm 0.2$  mJ/pulse laser irradiation (~60 min.). The legend depicts concentration of NPs as a mass percent of the toluene solution.

both before and after laser irradiation, displaying that, although small, the differences in absorbance before and after laser irradiation were outside of the error of the instrument, suggesting that irradiation time could potentially factor into the linear response of the detected PA pressure. Therefore, when subjecting  $Fe_3O_4$  NPs to laser irradiation during PA measurements, measurements were made as quickly as possible while still maintaining the 64 measurement average, 10 averages per concentration/energy combination, measurement parameters of the oscilloscope. This amounted to roughly 5 minutes of exposure to the 10 Hz laser pulses, or 28.8 µs of irradiation (approximately 3,600 pulses, at 8 ns each). While this will have the NPs exposed to laser energy for longer than was required to observe a change in the UV-Vis absorbance at 532 nm, it is as fast as the measurements can be collected without sacrificing the signal-to-noise ratio of the PA measurements.



Figure 5-3: Absorbance at 532 nm as measured by UV-Vis spectroscopy. The dependence upon concentration of the Fe<sub>3</sub>O<sub>4</sub> NP solutions was linear both before and after irradiation by  $3 \pm 0.2$  mJ/pulse laser light for 60 minutes.

Concentration (m/m % Fe <sub>3</sub> O <sub>4</sub> to toluene)	Abs. at 532 nm before irradiation	Abs. at 532 nm after irradiation
0.00032%	n/a	$0.01668 \pm 0.00007$
0.00634%	$0.04705 \pm 0.0000$	$0.05901 {\pm} 0.00008$
0.01590%	0.17402±0.00009	0.14122±0.00010
0.03170%	0.46865±0.00010	0.35927±0.00011

Table 5-1: Absorbance at 532 nm for Fe<sub>3</sub>O<sub>4</sub> NPs

# Section 5.3: Dependence of PA Waveform on Fe<sub>3</sub>O<sub>4</sub> NP Conc. and Laser Energy

The Fe<sub>3</sub>O<sub>4</sub> NP PA waveforms (Figure **5-4**) show many of the same features encountered with AuNPs. There is a sharp rise in peak intensity, a shallower trailing edge, and small fluctuations at or below the pressure of the initial baseline upon the return of the trailing edge to the baseline. The appearance of the peak is once again around 0.100 ms. The Fe<sub>3</sub>O<sub>4</sub> NP samples produced PA signals quite well up to 18 mJ/pulse incident light, where the peak shape changed significantly (Fig. **5-4f**). Instead of sharp peaks, the peaks were rounded, and occasionally flattened for several tens of



Figure 5-4: Full PA waveforms for  $Fe_3O_4$  NPs displaying changes in peak height and shape across concentration of the NPs (m/m %). Applied laser energy increased from a.-f. as follows: 3, 5, 8, 12, 15, and 18 mJ/pulse, with standard deviations 0.2, 0.3, 0.7, 0.5, 0.6, and mJ/pulse, respectively. Each waveform is the average of ten measurements made at each concentration and laser energy combination. microseconds. This was not observed in the most concentrated sample at that energy.

microseconds. This was not observed in the most concentrated sample at that energy.

It was observed that the minimum energy required to produce small peaks and bumps in the trailing edges of the  $Fe_3O_4$  NP signals was lower than it was for AuNPs. This makes intuitive sense, as the  $Fe_3O_4$  solutions are less optically dense than the AuNP solutions. With lower optical density should come a lower peak signal, and that is observed here. The peak  $Fe_3O_4$  signal is 75-80 kPa, while the peak signal for AuNPs is around 100 kPa. It is hypothesized that larger peak signals mask some of the smaller bumps and peaks in tailing edge, so with less intense peak signals it should be easier to observe these small bumps at lower energy.

The waveforms produced by AuNPs did not show steady changes with increasing laser energy, and so it encourages one to look at a similar comparison for  $Fe_3O_4$  NPs. In Figure 5-5, it can be seen that some peculiar trends are observed. To start, the control toluene solution produces no noticeable PA signal from 3 - 12 mJ/pulse (Fig. 5-5a), however at 15 mJ/pulse a peak is observed. It does not have the sort of smooth rising edge observed with NP-containing solutions, however it is a peak nonetheless, with the maximum occurring later than usual at roughly 0.15 ms. Increasing the laser energy to 18 mJ/pulse (Fig. 5-5b) reveals that the control peak occurs at roughly the same time following the trigger as it did with 15 mJ/pulse, however it is more than 2 orders of magnitude more intense (12,000 Pa compared to 80 Pa). As a result the 15 mJ/pulse peak is barely visible at the scale necessary to see the 18 mJ/pulse peak. These signals at 15 and 18 mJ/pulse could be related to the cuvette. As the cuvette was reused it became more difficult to clean, and there could have been residual NPs remaining on the cuvette that caused toluene to have a signal where it otherwise did not. Furthermore, repeated use

of the same cuvette for months on end eventually caused optical imperfections in the surface, which themselves could have been giving rise to a PA signal at sufficiently high laser energy. Simply replacing the cuvette periodically could prevent these problems from plaguing future measurements

The PA signal produced by the toluene control with 18 mJ/pulse irradiation can be seen in Figure **5-6a**. It is several orders of magnitude greater in strength than the signals produced by the control at lower laser energy levels, yet the rising slope of the peak is not as smooth as the peaks in Fe<sub>3</sub>O<sub>4</sub> NP-containing solutions. At approximately 75% of the peak maximum, the slope changes such that the final 25% of the peak height rises more slowly, suggesting that the typical PA signal production mechanism is not being observed. It is possible that the signal is being produced elsewhere in the cell as instrument background due to the absorption of laser light that is not attenuated by the sample solution. In the middle concentration ranges, the 18 mJ/pulse PA waveforms were prone to rounded off PA peaks.

With the 0.03170% solution (Fig. **5-6e**), the PA waveforms produced at 12, 15, and 18 mJ/pulse laser irradiation overlapped from start to finish. The rising edge slope, peak, trailing edge, and baseline signal of all three waveforms overlapped well, which is in contrast to the 0.01590% solution at 15 and 18 mJ/pulse, where only the peak intensity is overlapping, suggesting that the mechanism preventing the signal from increasing at higher energies is different for the most concentrated solution from the next most concentrated one. The peak intensity of the overlapping waveforms is less for the less concentrated solution than it is for the highest concentration, also suggesting that the mechanism is different.



Figure 5-5: Full PA waveforms of  $Fe_3O_4$  NPs across laser energy domain. Nanoparticle concentration increased from a.-f. as follows: 0.00% (m/m  $Fe_3O_4$  to toluene), 0.00%, 0.0032%, 0.0063%, 0.0159%, and 0.0317% . Each waveform is the average of ten measurements made at the same concentration and laser energy combination. Standard deviations of laser energy pulses are 0.2, 0.3, 0.7, 0.5, and 0.6 mJ/pulse for 3, 5, 8, 12, 15, and 18 mJ/pulse, respectively.

Looking at the NP-containing solutions, overlaying the PA waveforms for a single solution at different energy levels reveals little overlap as energy is increased except for with the most concentrated solution—a result similar to what was observed with the AuNPs. In Figure 5-5f, the peak pressure from 12 - 15mJ/pulse does not appreciably change. This was observed with the AuNPs as well, suggesting that there is some mechanism preventing the detector from picking up the entire signal produced in these conditions.

## Section 5.4: Linear Response of PA Pressure to Fe<sub>3</sub>O<sub>4</sub> Conc. and Laser Energy

As was done with the AuNPs, the focus now turns to the magnitude of the peak maxima. Examining the PA pressure peak produced by  $Fe_3O_4$  NPs showed better linear increase in peak intensity as concentration was increased (Figure **5-6** and Table **5-2**). The optical density of these solutions at 532 nm was overall less than the optical density of the AuNP solutions tested, lending credence to the notion that the instrument is better suited for such conditions. However, these results are not without their aberrations. While there did not appear to be saturation of the peak height, the 0.016% solution had a peak intensity at 15 mJ/pulse that was, within error, identical to the peak intensity at 18 mJ/pulse. This phenomenon was also observed in the most concentrated solution measured, 0.032%, with the signal produced at 12 mJ/pulse also having the same intensity as the 15 and 18 mJ/pulse signals. It is possible that the peak intensity observed with these 3 laser energy levels for the 0.032% Fe<sub>3</sub>O<sub>4</sub> NP solution (~75 kPa) is near the limit of the PA instrument, however this does not explain the overlapping peak intensities found with 15 and 18 mJ/pulse in the 0.016% solution, as the overlap observed with this

solution occurred at a peak intensity around ~53 kPa. It is possible that the limited number of nanoparticles in the 0.0159% solution compared to 0.03517% could not absorb the increased flux well enough to produce a different signal, however if this were the case the minimum energy needed to achieve maximum signal would go down as NP concentration goes down. We see the opposite, potentially due to some kind of optical density filtering where the origination point of the signal is changed at high concentration.



Figure 5-6: Fe<sub>3</sub>O<sub>4</sub> NP PA signal dependence on concentration across different laser energies. Each point is the average of ten measurements made at each concentration and laser energy combination with the standard deviation from the measurements depicted with bars. Standard deviations of laser energy pulses are 0.2, 0.3, 0.7, 0.5, and 0.6 mJ/pulse for 3, 5, 8, 12, 15, 18 mJ/pulse, respectively.

Applied Laser energy (mJ/pulse)	Linear equation	$R^2$
3	$y = (2.05 \pm 0.20)e7x - 270$	0.9724
5	$y = (3.64 \pm 0.16)e7x - 100$	0.9942
8	$y = (8.69 \pm 0.44)e7x - 400$	0.9924
12	$y = (2.29 \pm 0.15)e8x - 1700$	0.9878
15	$y = (2.48 \pm 0.27)e8x + 3500$	0.9664
18	$y = (2.11 \pm 0.17)e8x + 3800$	0.9800

Table 5-2: Linear best fit of Fe<sub>3</sub>O<sub>4</sub> PA pressure peaks vs. concentration

The linearity of the PA signal dependence on laser energy is less clearly defined than the linear response with respect to concentration. Figure 5-7, shows the peak PA signal in the laser energy domain (with Table 5-3 displaying the linear best fit equations to the data sets). There appears to be a tendency towards an exponential increase in PA signal with laser energy; however the signal intensity appears to stop increasing above 70 – 80 kPa. That observation applies to the most concentrated solution; however the next most concentrated solution had the same sort of leveling off at 60 kPa.

The peak in PA waveform for 0.01590% occurred at roughly the same laser energy as it did for the most concentrated sample (12-15 mJ/pulse), yet it is 10 - 20 kPa less intense. The cause of this phenomenon is difficult to ascertain, as less concentrated samples do not exhibit such behavior. On the note of less concentrated solutions, a PA peak is observed in the control toluene solution at 18 mJ/pulse that is equal to the signal produced by the least concentrated NP-containing solution at the same energy. So clearly there is some kind of PA pressure signal being produced by the instrument itself. There are few components of the instrument which are irradiated by the laser, and the primary one of interest here is likely the cuvette holder arm. The most secure mounting arrangement was determined to be one that put the clamp arm in the path of the laser, which could be absorbing small amounts of the transmitted laser energy, as the arm is not 100% reflective at 532 nm, and therefore producing a small signal on its own. This suggests that the cuvette holder could be producing a PA signal. This signal is rather weak in comparison to the signals produced by the more concentrated solutions at high energy, so this phenomenon is likely coincidental and not causal of the different peak PA pressure levels at 60 and 70-80 kPa. It seems as if the maximum achievable PA pressures



are only just achieved at 12 mJ/pulse, so perhaps higher energy levels should be avoided

Figure 5-7:  $Fe_3O_4$  NP PA signal dependence on laser energy across different concentrations. Laser energy increases (from a.-f.): 3, 5, 8, 12, 15, and 18 mJ/pulse with standard deviations of 0.2, 0.3, 0.7, 0.5, and 0.6 mJ/pulse, respectively. Each point is the average of ten measurements made at each concentration and laser energy combination, with the standard deviation of those measurements displayed with bars.

10

Laser Energy (mJ)

15

20

0.00

0

5

Fe <sub>3</sub> O <sub>4</sub> NP Concentration (m/m %)	Linear Best Fit	$\mathbf{R}^2$
0.00032%	$y = 0.230 \pm 0.060 x - 0.670$	0.7768
0.00634%	$y = 1.44 \pm 0.15 x - 5.61$	0.9592
0.01590%	$y = 3.32 \pm 0.64 x - 9.35$	0.8721
0.03170%	$y = 5.09 \pm 1.08x - 7.72$	0.8464

Table **5-3**: Linear best fit of Fe<sub>3</sub>O<sub>4</sub> PA pressure peaks vs. energy

The PA signal observed in blank solutions (Figures **5-6** and **5-7**) is likely due to a small PA signal produced by the cuvette mount. The mounting arrangement for the cuvette places the clamp arm post in the laser beam path in order to align the clamp arm opposite the piezoelectric detector to achieve a firm mount against it. (See Figure **3-6**.) The post is mechanically connected to the piezoelectric *via* the cuvette and the clamp arm. The post itself is made of aluminum and should be mostly reflective to 532 nm light,

but the potential exists for a PA signal to be developed by the post. The mechanical connection to the detector allows the signal to be detected if the laser energy impinging upon the post is sufficiently high. Altering this alignment to move the post out of the beam path without sacrificing the integrity of the cell mount should be attempted. Alternatively, a mirror could be placed in the small gap between the cuvette and the clamp arm post to direct the beam towards a beam dump, however there may not be adequate space available in the current arrangement in which to fit the mirror.

### Section 5.5 Calculating the Instrument Constant

Having acquired the necessary data to determine the instrument constant for  $Fe_3O_4$  NPs using equation 3-6:

$$m = \frac{kE\beta\varepsilon v^2}{C_p\sqrt{r}R^{3/2}} \to k = \frac{mC_p\sqrt{r}R^{3/2}}{E\beta\varepsilon v^2}$$
3-6

where laser energy changes from data set to data set, we find that the PA peak pressure responds in a more linear fashion to changing NP concentration than it does to laser energy. Therefore *k* for Fe<sub>3</sub>O<sub>4</sub> was calculated from the slope data in Figure **5-6**, giving the results in Table **5-4**. The average *k* was  $0.69 \pm 0.29$ , although it changed noticeably from energy to energy, indicating that, just as with AuNPs, *k* is dependent upon laser energy. *k* at each energy is, on average,  $53 \pm 10$  % less for AuNPs than it is for Fe<sub>3</sub>O<sub>4</sub> NPs, which could be due to a decrease in efficiency of the instrument as a result of acoustic saturation of the instrument with AuNPs. Furthermore, physical processes apart from photothermal lensing could be impacting the detected signal magnitude, which would artificially change the efficiency of the instrument. Despite this difference, as was found with the AuNPs, the maximum *k* was found at 12 mJ/pulse. After accounting for error, *k* at 12 mJ/pulse is just around 1 for  $Fe_3O_4$  NPs, indicating excellent instrument efficiency. With the instrument proving to be most efficient at detecting acoustic energy at 12 mJ/pulse, the notion that higher energy levels exceed the detection capacity of the instrument or exhibit destructive interference is supported.

Laser energy (mJ/pulse)	k (dimensionless)
3	0.37±0.04
5	0.39±0.03
8	$0.58{\pm}0.05$
12	$1.03 \pm 0.09$
15	0.88±0.12
18	$0.62 \pm 0.08$

Table 5-4: Instrument Constant for Fe<sub>3</sub>O<sub>4</sub> NPs with varying excitation energy

If k is used to normalize the PA peak pressure in Figure 5-7, the linear relationship of the data is improved for  $Fe_3O_4$  NPs just as it was for AuNPs. The results of such normalizing are found in Figure 5-8, and while the linear response improves with



Figure 5-8: PA peak pressure for  $Fe_3O_4$  plotted against laser energy following normalizing with *k* obtained from the slope of PA peak pressure versus NP concentration. Standard deviations for 3, 5, 8, 12, 15, and 18 mJ/pulse are 0.2, 0.3, 0.7, 0.5, and 0.6 mJ/pulse, respectively.

Fe <sub>3</sub> O <sub>4</sub> NP conc. (m/m %)	Line of Best Fit	$R^2$
0.0032 %	$y = 0.220 \pm 0.070 x - 0.23$	0.7107
0.00634 %	$y = 1.43 \pm 0.19 x - 3.74$	0.9314
0.01590 %	$y = 3.11 \pm 0.73 x - 1.84$	0.8200
0.03170 %	$y = 4.34 \pm 0.86 \ x - 10.6$	0.8635

Table 5-5: Lines of best fit for k-normalized PA pressure vs. Laser Energy

higher energy, the improvement is minimal with lower concentration samples. The  $R^2$  values can be found in Table 5-5. It is found that at 18 mJ/pulse irradiation energy, the linear relationship of the normalized PA pressure deteriorates; this lends further credence to the notion that high energy is unsuitable for the instrument as assembled.

# Section 5.6 Conclusions

Iron oxide nanoparticles exhibited many of the same PA waveform patterns observed in gold nanoparticles, however they seemed to be more stable under irradiation, and provided better linear fits in plots of PA pressure versus NP concentration. They did share with AuNPs a lack of linear PA response to laser energy and a similar maximum viable irradiation energy level (18 and 21 mJ, respectively). Yet another similarity between the two analytes was the change in waveform shapes. Specifically both NP types experienced changes in the tailing edge of the waveform, if a change in waveform shape was experienced at all. These changes occurred at high concentration and energy. The Fe<sub>3</sub>O<sub>4</sub> NPs were 3 times larger in diameter than the AuNPs and did not exhibit plasmon resonance, suggesting that other types of NPs can successfully be measured using this system. With unusually high temperatures occurring with both NP materials measured here, the desire to detect microscale boiling directly, and potentially quantify it, arise.
Such methods do exist, and a proposed method to incorporate such methods into this instrument are discussed in Chapter 6.

# **Chapter 6**

# **Calculating Temperature from the Photoacoustic Pressure**

### Section 6.1 Introduction

We have found a wide variety of photoacoustic experiments and measurements in the literature; however these reports do not often discuss the relationship between PA pressure and the change in temperature of the absorbing material. When the relationship is mentioned, and even when the determination of temperature is the goal of the work, the approach used is poorly reported. Often, a known temperature is used to calibrate the PA instrument signal, and the temperature produced by the photothermal substance is determined in that way. In our case the  $\Delta T$  is expected to be large enough that such temperatures are not easily attained otherwise. In an effort to determine just how hot these nanoparticles can get under laser irradiation to both fill this gap in the literature and supplement other work performed in the Lear Group, various equations were explored to link PA pressure to temperature. Description of the equations and the results obtained from them are explained here and then compared to theoretical values for the NP temperature to gauge the validity of the selected approach.

### Section 6.2 Theoretical Calculation of Heat and Change in Temperature per NP

Before working with the PA data, it seems appropriate to determine the theoretical heat release given the absorptive properties of the nanoparticles. This can be done by assuming that all absorbed power is converted into photothermal heat—a reasonable assumption given the poor fluorescent properties of these nanoparticles. The

maximum theoretical absorbed power per nanoparticle,  $P_{abs}$  (Equation 6-1), can be found using the cross sectional area of each nanoparticle (cm<sup>2</sup>/np), the irradiation power density (W/cm<sup>2</sup>), and the absorption coefficient (dimensionless, Equation 6-2). The absorption coefficient can be determined using the refractive index of the medium surrounding the nanoparticle ( $\eta_m$ ), radius of the nanoparticles, real ( $\epsilon$ ') and imaginary ( $\epsilon$ '') dielectric constants of the nanoparticle material, and the wavelength of light are known.  $Q_{abs}$  was 0.182 for AuNPs and 0.170 for Fe<sub>3</sub>O<sub>4</sub> NPs.

$$P_{abs} = A_{NP} I_0 Q_{abs} ag{6-1}$$

$$Q_{abs} = \frac{24\pi^2 r_{np} \eta_m^2 \varepsilon_{np}}{\left(2\eta_m + \varepsilon_{np}'\right)^2 \lambda}$$
 6-2

There are two proposed mechanisms for heat release from the nanoparticles: convection heating and blackbody radiation. [40] These processes can be generalized for spherical objects with radius *r*. The thermal convection is dependent upon the heat capacity,  $C_p$ , of the surrounding medium and the temperature difference between the sphere and the surrounding medium (presumed to be at room temperature). Blackbody radiation is independent of sphere and surrounding medium composition. It involves surface area, A, and absolute temperature, T, of the sphere and the Stefan-Boltzmann constant,  $\sigma_{sb}$ . Together, this yields an equation (6-3) which relates a given heat flux to a steady state temperature (T).

$$\frac{Q}{NP} = 4\pi r C_p (T - T_{RT}) + \sigma_{sb} A T^4$$
 6-3

The temperature can thus be solved numerically to determine the heat released per NP and the change in nanoparticle temperature accompanying said heat.

If the  $P_{abs}$  calculated for the appropriate nanoparticles is used as the watts emitted per nanoparticle in equation 6-3, ranges for heat release and changes in temperature are calculated for both nanoparticle compositions ( $10^1-10^2$  nW/np and  $10^0-10^2$  Kelvin/np, see Table 6-1). These temperatures should be sufficient to help catalyze reactions in the environment immediately surrounding the nanoparticles.

Irradiation Energy	Aul	NPs	Fe <sub>3</sub> O <sub>4</sub> NPs		
(mJ/pulse)	nW/np		nW/np $\Delta T/n$		
3	17.4	9.70	139	26.5	
5	29.0	16.2	231	44.2	
8	46.4	25.9	370	70.8	
12	69.4	38.8	553	106	
15	86.9	48.6	693	132	
18	104	58.0	832	159	
21	122	68.0	971	186	

Table 6-1: Theoretical Heat Release and Change in Temperature per NP

The P<sub>abs</sub> can also be used to determine the anticipated change in temperature of the solvent in the laser beam using equation 6-4, where  $C_p$  is the heat capacity of toluene at constant pressure,  $N_{np}$  is the number of nanoparticles in the irradiated volume, and  $n_{solv}$  is the moles of toluene in the irradiated volume (obtained using the volume of the laser beam in solution and the density of toluene). Reasonable results are achieved with the change in temperature of the solvent being effectively zero  $(10^{-11} - 10^{-9} \text{ K}, \text{ Tables 6-2} \text{ and 6-3})$ , which closely matches the observation that the cuvette, immediately following laser irradiation, was not noticeably warmer to the touch.

$$\frac{Q}{NP} = \frac{C_p \Delta T n_{solv}}{N_{np}}$$
 6-4

Irradiation Energy	$\Delta T$ of irradiated solvent (pK)			
(mJ/pulse)	0.0057%	0.0114%		
3	56.8	114		
8	152	303		
12	227	453		
15	284	568		
18	341	682		
21	398	796		

Table 6-2: Theoretical Solvent Temperature Change with AuNPs

Table 6-3: Theoretical Solvent Temperature Change with Fe<sub>3</sub>O<sub>4</sub> NPs

Irradiation Energy	$\Delta T$ of irradiated solvent (pK)				
(mJ/pulse)	0.0032%	0.0063%	0.0159%	0.0317%	
3	110	220	552	1,100	
5	183	367	920	1,830	
8	294	588	1,470	2,940	
12	439	878	2,200	4,390	
15	550	1,100	2,760	5,500	
18	661	1,320	3,310	6,610	

### Section 6.3 Experimental Calculation of Heat and Change in Temperature per NP

The discussion of the temperature change that led to the photoacoustic pressure measured begins with an adjustment to make sure the pressure used is truly the pressure inside the cuvette. The instrument constant can be combined with the measured PA pressure to obtain the PA pressure impinging on the cuvette wall. The nanoparticles at the cuvette wall are not being irradiated and therefore do not contribute to the PA pressure *via* heat release. By using the pressure measured at the wall, the pressure at the laser beam edge can be determined. These two steps are shown at once in equation 6-5, where  $P_{A,r1}$  and  $P_{A,r2}$  are the PA pressure at the wall and at the laser beam edge, respectively, where r1 and r2 are the distance from the laser beam center to the cuvette wall and the laser beam edge, respectively.

$$\frac{P_{A,r1}}{k}\sqrt{r1} = P_{A,r2}\sqrt{r2}$$
 6-5

If one assumes that the pressure experienced by toluene at the laser beam edge is isothermal, the isothermal compressibility of toluene ( $\beta_T$ , [41]) can be used to determine the change in volume experienced by the solvent at that point (Equation 6-6, where V = irradiated volume). This change in volume can be related to a change in temperature of the irradiated volume as a whole *via* the volumetric expansion coefficient of toluene ( $\beta$ , Equation 6-7).

$$\Delta V = \Delta P \beta V \tag{6-6}$$

$$\Delta T = \frac{\Delta V}{V\beta}$$
 6-7

The heat to obtain this temperature change was provided by the nanoparticles in the irradiated volume, so equation 6-4 is used to determine the heat per nanoparticle. Equation 6-3 is then used to determine the change in temperature per nanoparticle.

In summary, using equations 6-3 through 6-7 the PA pressure at the cuvette wall is transformed in the following sequence into change in temperature per nanoparticle: pressure at wall  $\rightarrow$  pressure at laser beam edge  $\rightarrow$  heat absorbed by toluene in the laser beam volume  $\rightarrow$  heat released per nanoparticle  $\rightarrow$  temperature change experienced per nanoparticle.

$$P_{A} = \frac{kE_{0}\varepsilon\beta v^{2}}{c_{p}\sqrt{rR^{3/2}}} \rightarrow \Delta V = \Delta P\beta_{T}V \rightarrow \Delta T = \frac{\Delta V}{V\beta} \rightarrow \frac{Q}{np} = \frac{c_{p}\Delta T n_{toluene}}{N_{np}\tau_{pulse}} \rightarrow \frac{Q}{np} = 4\pi r_{np}\Delta T + \sigma_{SB}A_{np}T^{4}$$

The results of these calculations are presented in Tables **6-4** and **6-5**. Units in these tables are Watts per nanoparticle and MegaKelvin. Examination of the obtained heat and temperature values reveals that each nanoparticle supposedly achieved temperatures in the millions of Kelvin. This does not make intuitive sense, and either one or both of the following is likely the cause of this result: the pressure to temperature calculation in the solvent is incorrect, or an artificially large photoacoustic pressure being detected by the piezoelectric device. The latter could be caused by micro-boiling of the solvent in the environment immediately surrounding the nanoparticles. This could cause the pressure to be due to gas evolution in addition to thermal propagation. Furthermore, AuNPs exhibit

Excitation Energy	0.0057%	% AuNP	0.0114% AuNP		
(mJ/pulse)	W/np	MK	W/np	MK	
3	723	5.62	697	5.56	
8	2130	7.35	1860	7.11	
12	3320	8.22	2780	7.87	
15	4030	8.63	3480	8.32	
18	6370	9.67	4180	8.71	
21	7530	10.1	4880	9.05	

Table 6-4: Thermal energy released and change in temperature per AuNP

Table 6-5: Thermal energy released and change in temperature per Fe<sub>3</sub>O<sub>4</sub> NP

Excitation Energy	0.000317 % Fe <sub>3</sub> O <sub>4</sub>		0.00634 % Fe <sub>3</sub> O <sub>4</sub>		0.0159 % Fe <sub>3</sub> O <sub>4</sub>		0.0317 % Fe <sub>3</sub> O <sub>4</sub>	
(mJ/pulse)	W/np	MK	W/np	MK	W/np	MK	W/np	MK
3	68.8	1.82	135	2.16	219	2.44	300	2.64
5	141	2.18	334	2.71	499	2.99	489	2.98
8	228	2.46	612	3.15	689	3.25	800	3.37
12	334	2.71	747	3.31	1000	3.57	1200	3.72
15	764	3.33	1610	4.01	2000	4.24	1450	3.91
18	467	2.95	1930	4.20	1490	3.94	1220	3.74

fragmentation under high laser fluence at the plasmon resonance, so the energy released during fragmentation of the AuNPs could be artificially boosting the PA pressure. [42] The pressure detected revealed a change in temperature in the millions of Kelvin for  $Fe_3O_4$  NPs, as well (Table 6-2). Once again, this temperature is seemingly higher than is physically possible, indicating that the proposed calculation method is in need of further work.

An alternative method of determining the change in temperature in the solvent was attempted with the Grünieissen parameter,  $\Gamma$  (Equation 6-6). [43, 44] This dimensionless parameter directly relates the change in pressure experienced by a system resulting from a change in temperature. In comparison to the previous calculation route, the Grüneissen parameter omits the calculation of  $\Delta V$ .

$$\Gamma = \frac{\beta v^2}{c_p} = \frac{V}{c_V} \left(\frac{\Delta P}{\Delta T}\right)_V$$
6-6

np

$$\underline{\operatorname{Previously}}: P_{A} = \frac{kE_{0}\varepsilon\beta v^{2}}{C_{p}\sqrt{r}R^{3/2}} \rightarrow \underline{\Delta V} = \underline{\Delta P}\beta_{\underline{\#}}V \longrightarrow \underline{\Delta T} = \frac{\underline{\Delta \Psi}}{\underline{\Psi}\underline{\Phi}} \rightarrow \frac{Q}{np} = \frac{C_{p}\Delta Tn_{toluene}}{N_{np}\tau_{pulse}} \rightarrow \frac{Q}{np} = 4\pi r_{np}\Delta T + \sigma_{SB}A_{np}T^{4}$$

$$\underline{\operatorname{Using Grüneissen Parameter}}: P_{A} = \frac{kE_{0}\varepsilon\beta v^{2}}{C_{p}\sqrt{r}R^{3/2}} \rightarrow \Delta T = \frac{C_{p}V\Delta P}{C_{V}\beta v^{2}} \rightarrow \frac{Q}{np} = \frac{C_{p}\Delta Tn_{toluene}}{N_{np}\tau_{pulse}} \rightarrow \frac{Q}{np} = 4\pi r_{np}\Delta T + \sigma_{SB}A_{np}T^{4}$$

Here, many of the same constants used in the conversion of pressure to temperature are encountered once more ( $\beta$ ,  $\nu$ ,  $C_p$ , and V), with  $C_V$ —the isochoric (constant volume) heat capacity of the solvent added in. The isochoric heat capacity is not routinely determined at room temperature, however it can be calculated using equation 6-7, which utilizes the constant pressure heat capacity, volume and temperature

of the solvent, thermal expansion coefficient ( $\alpha$ ), and the isothermal compressibility ( $\beta_T$ ). This calculation reveals C<sub>V</sub> to be very similar to the C<sub>p</sub> (155.55 J/(mol\*K) and 155.96 J/(mol\*K), respectively).

$$C_V = C_p - \frac{VT\alpha^2}{n_{solv}\beta_T}$$
 6-7

By bypassing the pressure to volume calculation (equation 6-6), it was presumed that the results for heat released and change in temperature per nanoparticle would be different than those values obtained previously, however they were of roughly the same order of magnitude, indicating that there is still a gap in the understanding of the link between pressure and temperature.

It must also be noted that there may be physical processes happening in the environment surrounding the nanoparticles which are not accounted for by these calculations. The heat released, while logically not on the order of  $10^6$  K, is still theoretically sufficient to boil the solvent immediately surrounding the NPs. This would cause rapid short-lived, but potentially significant, changes in the volume of the irradiated volume. A change in volume does impact the pressure of a system, so this may contribute to abnormally high pressure readings. This may account for some of the seeming error found with the above calculations. A method to attempt to quantify the degree of any micro-scale boiling should be explored, and preliminary work exploring this with the present instrument is presented in Section 7.2.

## Section 6.4 Conclusions

The linear relationship of photoacoustic pressure with nanoparticle concentration was good; however attempts to calculate the heat that led to the pressure detected have revealed a gap in information. It is possible that the presented method to calculate the heat flux per nanoparticle is making incorrect assumptions with respect to how heat produces pressure. Depending on the severity of the correction needed, the difference between calculated and theoretical heat per nanoparticle could be accounted for. It is also worth investigating the possibility of the photoacoustic pressure being altered through non photothermal processes such as micro-scale boiling of the solvent immediately surrounding the nanoparticles.

## Chapter 7

## **Conclusions and Proposed Future Directions**

### Section 7.1 Instrument Utility

The construction of a new instrument should yield a piece of equipment that can be used repeatedly with little maintenance if one is to reap the benefits of avoiding costly commercial equipment. The instrument constructed here withstood repeated disassembly and assembly both between measurements of different analytes and also between measurements of the same analyte with different concentration or energy parameters. The instrument proved to be most efficient at 12 mJ/pulse for the transfer of acoustic energy from the solvent to the detector, and it is suggested that this excitation energy be used as the peak excitation energy in future studies.

While the instrument proved to be reliable given the present assembly, the design was developed such that light sources and sample containers could be easily switched in and out. This should be taken advantage of through the use of multiple different excitation wavelengths in the future. Being able to preferentially excite the plasmonic or non-plasmonic absorption bands of NPs will help to reveal the role of plasmonic resonance in photothermal heating. Furthermore, if such alternate light sources can be digitally controlled, alternate pulse durations should be explored to determine the effect on photothermal production. Longer pulses should lead to an increase in heat production if the system does not become saturated through heat exchange with the air surrounding Alternative sample cells could lead to the use of longer pathlength cells (hypothetically permitting the use of less concentrated solutions) or cells where the detector is located along the same axis as the laser beam, permitting the detection of spherically-sourced acoustic waves in more optically dense solutions. [22]

### Section 7.2 Photothermal Lensing and Preliminary Results Thereof

Under high fluence or high concentration, the PA signal tail shape was observed to contain shoulders and peaks not found in low fluence or low concentration measurements. Additionally, the PA pressure leads to unreasonably large releases of heat per nanoparticle. One potential explanation for these differences in the waveforms and the unusual thermal calculations could be that energy absorbed by the nanoparticles was being used in ways that were not purely thermal dissipation: micro-scale boiling of the solvent, for example. Short lived boiling may have been detected by the piezoelectric detector due to the pressure pulses created by bubble formation and collapse, akin to a process known as cavitation, where an ultrasonic wave causes bubbles to form and collapse, releasing immense amounts of heat. [45] In order to check for this behavior, a separate technique is needed. Thermal lensing is one such technique which measures heat due to a change in refractive index that deflects a second laser passed through the sample. [46] At the same time, this probe beam would be sensitive to scattering from any bubbles formed. The appearance of these bubbles would be manifest in a discontinuity of a graph of probe intensity versus pump power.

To test for boiling, a system was set up where the previous Nd:YAG arrangement was modified such that the PA signal triggered the oscilloscope to measure the intensity of a laser pointer aimed through the cuvette, as detected by the photodiode that was previously being used to trigger the oscilloscope off of the Nd:YAG pulse (Figure 7-1).

In doing so, our hypothesis was that any boiling or thermal lensing effect would decrease the energy from the laser pointer detected by the photodiode—either due to scattering or deflection of the beam. A black tube with an optical filter on one end (so as to be opaque to the scattered 532 nm light from the laser) was mounted over top of the photodiode to prevent the Nd:YAG's second harmonic output from reaching it and giving a false signal. The laser pointer, so as to ensure constant energy output from its aperture, was wired to be driven by a function generator (Mastech SFG-1002 Function Generator, 2 MHz). The function generator was set to 0.05 Hz output in order to keep the laser pointer consistently powered on for ~10 seconds, which was enough time to collect a well-averaged signal from the photodiode by the oscilloscope. (Averaging performed over 64 samples at 10 Hz required 6.4 seconds). By using the function generator, we were able to avoid laser pointer energy output loss due to draining batteries.



Figure **7-1**: a. Nd:YAG laser, b. beam path, c. photodiode, d. PA cell/detector, e. preamplifier, f. oscilloscope, g. 647 nm laser pointer, h. function generator.

Initial studies started at low laser pump energy (3 mJ/pulse), collecting measurements of 10 samples (each an average of 64 measurements) at each laser energy before increasing it incrementally up to 30 mJ/pulse. Data set "Low to High" in Fig. **7-2** shows that the measured laser pointer energy decreased exponentially across increasing laser energy. This result was promising, however to test for its validity, the thermal lensing experiment was repeated starting at high energy and gradually lowering it (data set "High to Low" in Fig. **7-2**). Here it can be seen that the exponential relationship is not maintained, suggesting that either the thermal lensing detection scheme requires modification, or that the 647 nm laser pointer as driven by a function generator is not a reliable probe beam.



Figure 7-2: Probe beam intensity versus pump beam energy. An exponential fit is found with increasing laser energy ("Low to High") but is not found with decreasing laser energy ("High to Low").

Future work should expand upon this curious discrepancy by either using a more powerful (and steadier) probe beam or by using a different probe schematic. A suggested change to the schematic is a longer probe beam path between the solution and the photodiode, as a longer beam path after the solution would exaggerate any beam deflection. The use of a longer pathlength sample cell could improve the sensitivity of the deflection to any boiling, as well, however pump beam attenuation through such a cell would be of concern, thereby limiting the optical density of the solutions used.

### Section 7.3 Effect of Analyte on the Signal Shape and Proposed Future Analytes

This initial work has shown the instrument to be reliably linearly responsive to NP concentration with two different NP compositions. The observation that the PA waveform peak occurs at the same time after the laser pulse with both NP compositions suggests that the peak detection is not dependent on the presence of a plasmon resonance band. Next, the photothermal dependence upon nanoparticle size, shape, and composition should be explored further, as all have been shown to control the optical properties of nanoscale materials. A good first analyte to use for future work would be copper neodecanoate NPs, which have, much like AuNPs, proven to be useful in biological photothermal work regarding oncological studies. [20] However this should be performed if the copper neodecanoate NPs can be made on the same size scale as the AuNPs and Fe<sub>3</sub>O<sub>4</sub> NPs used in this work. Alternatively the AuNPs and Fe<sub>3</sub>O<sub>4</sub> NPs could be made larger and then compared to copper neodecanoate NPs as available-regardless, a comparison of NP composition should be made across similar spherical radii. Determining the instrument constant, k, and its dependence upon NP composition may reveal if the instrument is preferentially efficient towards heating that is independent of plasmonic resonance.

Each NP composition used should also be explored at different sizes, as larger sizes should result in an increase in heat produced per NP due to the increased mass of each NP. Such studies would have to be carefully controlled for laser energy, though, as high light flux has been shown to fragment certain sizes of NPs. [42] A reasonable choice for such size studies would be citrate-stabilized AuNPs, as the size of these spherical NPs can be controlled systematically in synthesis. [47]

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