Implementation and Detection Optimisation of Gold Nanoparticles as Contrast Media in
Diagnostic Radiology

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Submitted by

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ABSTRACT

This project has focused on identifying any interesting properties, particularly from a physics perspective, that gold nanoparticles offer as the basis for a contrast agent in diagnostic radiology. The use of colloid, or nanoparticulate, contrast agents is not a new proposition (for example: early thorium-based contrast media), but in the last several decades iodinated compounds have become the standard due to their safety and relatively high radiopacity. Accordingly, contrast-aided procedures employing iodine are set to exposure parameters designed to optimally enhance the K-edge of that particular element (33.2 keV).

With improvements in chemical engineering, understanding of protein-cell interactions, and the ability to record measurements on a nanometre scale, there has been renewed interest in the use of nanoparticles for biological applications. Gold nanoparticles suspension represents a particularly attractive candidate for use in radiographic imaging. There are well documented synthesis procedures for gold nanoparticles. The element has a relatively large atomic number and is subsequently highly radiopaque. Gold particles can be bound to cell-specific targeting molecules. Gold as an element is bioinert and while the toxicity of nanoparticles is still debatable, gold is expected to have markedly lower toxicity than most other heavy elements.

In this project I have sought to determine the most practical X-ray imaging modalities for use of gold nanoparticle contrast media. Special emphasis has been placed on optimally detecting attenuated photons above the K-edge of gold (80.7 keV). The optimal energy spectrum to maximise relative contrast of gold nanoparticles with respect to normal tissues and conventional contrast media has been investigated by phantom study with radiographic images. These results are supported by quantitative simulations by Monte Carlo technique which further identify that the optimal tube potential for visualisation of gold nanoparticles is in the range of 120 to 140 kilovolts for most applications. Further study has considered the
implementation of a combined, double-layer detector system to capture simultaneous subtraction images which would benefit from the relatively high K-edge of Au. Finally, experiments designed to shape the X-ray energy spectra transmitted from a source were conducted and applied to a novel subtraction imaging technique. Results from those experiments showed particular promise for maximising the detection of small quantities of Au in dual-energy subtraction imaging.

Attention has been given to nanoparticle-specific size-effects. These quantum confinement effects can subtly alter the attenuation of X-ray photons by very small clusters of atoms compared to bulk crystal structures. Measurements were completed using X-ray Absorption Spectroscopy and also in radiographic imaging with the aim of determining whether an optimal particle size should be selected to maximise image contrast in a radiographic image.
Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and ethics procedures and guidelines have been followed.

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<tr>
<td>AAS</td>
<td>Atomic Absorption Spectroscopy</td>
</tr>
<tr>
<td>ARPANSA</td>
<td>Australian Radiation Protection and Nuclear Safety Agency</td>
</tr>
<tr>
<td>AuNP</td>
<td>Gold Nanoparticle</td>
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<tr>
<td>BIP</td>
<td>Back-illuminated Photodiode</td>
</tr>
<tr>
<td>CHCl₄</td>
<td>Chloroform</td>
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<tr>
<td>CM</td>
<td>Contrast Medium/Media</td>
</tr>
<tr>
<td>CR</td>
<td>Computed Radiography</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>DSA</td>
<td>Dual-energy Subtraction Angiography</td>
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<tr>
<td>DQE</td>
<td>Detective Quantum Efficiency</td>
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<tr>
<td>DR</td>
<td>Direct Radiography</td>
</tr>
<tr>
<td>DSCT</td>
<td>Dual-Source Computed Tomography</td>
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<tr>
<td>ECF</td>
<td>Extracellular Fluid</td>
</tr>
<tr>
<td>EGSnrc</td>
<td>Electron Gamma Shower – Monte Carlo package based on EGS version 5.0 from the National Research Council of Canada</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
</tr>
<tr>
<td>GI</td>
<td>Gastro-intestinal</td>
</tr>
<tr>
<td>GOS</td>
<td>Gadolinium Oxysulphide</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>HAuCl₄</td>
<td>Chloroaauric Acid</td>
</tr>
<tr>
<td>HOCM</td>
<td>High Osmolar Contrast Medium</td>
</tr>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>HU</td>
<td>Hounsfield Units</td>
</tr>
<tr>
<td>HVT</td>
<td>Half Value Thickness</td>
</tr>
<tr>
<td>IPEM</td>
<td>Institute of Physics and Engineering in Medicine</td>
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<tr>
<td>IR</td>
<td>Image Receptor</td>
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<tr>
<td>kVp</td>
<td>Peak Kilovoltage</td>
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<td>LOCM</td>
<td>Low Osmolar Contrast Medium</td>
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<tr>
<td>mA</td>
<td>Milli-ampere (electrical current in thousandths of coulombs per second)</td>
</tr>
<tr>
<td>MC</td>
<td>Monte Carlo</td>
</tr>
<tr>
<td>NaBH₄</td>
<td>Sodium Borohydride</td>
</tr>
<tr>
<td>PAMAM</td>
<td>Polyamidoamine</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered Saline</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate), Acrylic, Perspex</td>
</tr>
<tr>
<td>PMT</td>
<td>Photo-multiplier tube</td>
</tr>
<tr>
<td>PSP</td>
<td>Photo-stimulable Phosphor</td>
</tr>
<tr>
<td>RDF</td>
<td>Radial Distribution Function</td>
</tr>
<tr>
<td>ROI</td>
<td>Region-of-Interest</td>
</tr>
<tr>
<td>RNG</td>
<td>Random Number Generator</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface-enhanced Raman spectroscopy</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise Ratio</td>
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<tr>
<td>SPECT</td>
<td>Single-photon-emission Computed Tomography</td>
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<td>SRCT</td>
<td>Synchrotron Radiation Computed Tomography</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
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<tr>
<td>ThO$_2$</td>
<td>Thorium Dioxide; aka Thoria, Thorina, Thorium(IV) Oxide</td>
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<td>XAFS</td>
<td>X-ray Absorption Fine Structure</td>
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<td>XANES</td>
<td>X-ray Absorption Near-edge Structure</td>
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<td>XAS</td>
<td>X-ray Absorption Spectroscopy</td>
</tr>
<tr>
<td>YAG</td>
<td>Yttrium Aluminium Garnet</td>
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PUBLICATIONS ARISING FROM THIS WORK:


Price A. Jackson, Moshi Geso. “Gold Nanoparticles as Contrast Media in Dual-energy Radiography: a Monte Carlo Study” published in conference proceedings and presented as poster at the TechConnect World Congress and Expo Anaheim, CA, 22-26 June 2010.


1. Introduction

1.1 Diagnostic Imaging

Since its first discovery, the use of radiation in imaging has undergone a steady evolution. There have been incremental improvements in resolution, reduction in patient radiation dose, and diagnostic specificity for particular pathologies. In the last century, its use has been extended from a handful of experimental labs to become a part of everyday life. This remarkable ability to see through an object and discover its internal components in a non-destructive fashion is vital in dozens of fields. Engineers, security personnel, astronomers all utilise the transmission or detection of high-energy photons in their trade. But no field has reaped greater benefit from the X-rays than diagnostic medicine.

In 1990, it was estimated that approximately 250 million X-ray examinations were performed annually in the United States (1). That figure is expected to have steadily grown over the last two decades. Radiographic imaging can be employed for a broad range of procedures, from identification of broken bones to the delineation of malignant tissues. The current generation of cutting-edge computed tomography scanners can reproduce three-dimensional representations of the entire body in a matter of seconds. These machines even have the ability to differentiate some tissues on the basis of their material composition (2). The gradual adaptation of this equipment gives physicians more powerful tools than would ever have been imagined when Roentgen first excited a barium platinocyanide screen in 1895 (3). And with each decade that passes, techniques that were thought to be beyond the limits of technology and practicality become the clinical standard. As a physicist and researcher the aim is the push these limits, while still working within the constraints of the medium.
1. Introduction

For all its wide-spread use, radiography does suffer from some inherent limitations. There are no optics in medical radiography. Lenses cannot be used to alter the angle of the X-ray beam in a hospital environment. As a result, radiography relies on shadow-type images. Even computed tomographic images represent a reconstruction of many different projections. X-rays emitted from a source are attenuated to varying degrees by tissues along a path to an image detector. The process relies on patients absorbing a certain amount of radiation which is representative of tissue composition, thickness, or density. The transmitted X-rays thereby represent information which, when divided over an array of small pixels, produces the familiar radiographic output image. A larger quantity of X-rays improves the statistical sampling at each pixel. However, with the understanding that radiation is harmful however, there is a balance with the acceptable radiation dose that can be given to a patient while still absorbing enough of the incident photons to produce a high-quality image (4).

There are also limitations in the engineering of X-ray equipment, in particular in efficiently translating the transmitted photons into information in the image receptor (IR). The IR must be able to capture a large quantity of the transmitted X-ray photons while still being able to differentiate small details of patient anatomy. Computed Tomography scanners are prone to artefacts due to the reconstruction process (particularly in using early simple backprojection algorithms) (5).

There are often techniques which can be used to overcome one limitation or another, but at the sacrifice on image quality or patient dose in a different respect. For example, image resolution can be improved by moving the image receptor or film away from the patient. This “air gap” technique allows the divergent X-ray beam to expand over a greater area before reaching the image receptor, thereby magnifying the structures in the recorded image. There are two problems with such an approach. First, the patient must receive a greater dose of radiation to maintain the same level of exposure at the film. Second, because an X-ray tube
emits a divergent beam of photons from a focal spot that has a finite area (as opposed to an infinitely small point in space), X-ray images suffer from some partial-shadowing, or penumbra, that reduces the resolution of small details. This effect becomes more pronounced when the distance between patient and image receptor is increased (4). The result is that, even though an image may be magnified by air gap technique, the recorded image may actually produce less detail. As such, the detection of subtle, fine features, particularly in a large volume of tissue, is often accomplished by the introduction of a highly-attenuating foreign substance: a contrast medium.

Ideally monoenergetic X-rays would be used in diagnostic imaging. By selecting photons of a single wavelength, the beam could be tuned to maximise the contrast produced by certain materials of interest in the body without requiring the patient to absorb a large quantity of low-energy photons that contribute considerably more to radiation dose than image quality. X-ray tubes inherently produce a broad range of photon energies. That is, for a selected potential difference, the photons exiting the tube will have a variety of wavelengths associated with a range of energies between 0 keV and the peak kilovoltage for the selected tube potential. In spite of a large evolution in terms of photon detection and computation algorithms, the process of forming radiation in a vacuum tube remains virtually unaltered since its advent over a century ago. This process of X-ray generation relies on the acceleration and sudden deceleration of electrons in an evacuated glass tube. The application of a very strong potential difference (in the range of 20 to 150 thousand volts) donates kinetic energy to the electrons, up to the selected peak kilovoltage (in kilo-electron volts, keV). Interaction between the accelerated electrons and tungsten atoms in the anode of the vacuum tube causes the release of energy in one of three forms: heat, Bremsstrahlung radiation, or characteristic radiation. The process of X-ray generation is relatively inefficient, losing over 90% of energy as heat which must be safely removed from the tube (6). More importantly, it
produces a beam of X-ray photons with an indiscriminate range of energies, many of which won’t be useful for imaging (see Figure 1.1). The spectral distribution of a beam may be altered to some degree by either increasing the peak tube potential or adding a filter material. However an idealized beam of monochromated, coherent x-rays can only be produced by a synchrotron.

![X-ray Energy Spectra](image)

**Figure 1.1:** X-ray energy spectra for combinations of tube potential and added filtration. Adjustments of these parameters affect beam penetrability and image contrast. Fine-tuning can improve resolution of radiopaque contrast media relative to anatomical structures.

In most cases, these limitations are unimportant. For an examination targeting a number of different anatomical features that vary in their elemental make-up, the broad emission spectrum from an X-ray tube is appropriate. A small amount of low energy photons will increase the dose beyond what is ideal, and a small amount of high energy photons will wash out the image reducing contrast. However peak region of X-rays, near the mode of the spectral curve, will fall into a range that provides good contrast for a number of anatomical
features with an acceptable absorbed radiation dose to the patient (7, 8). The effect on image quality and dose of the respective tails in the x-ray spectrum are negligible, especially when compared to the cost of implementing a more advanced technique for generating diagnostic-type radiation.

The second limitation, mentioned briefly above, is the desire to minimise patient radiation dose when producing images. X-ray images are produced by a finite number of electromagnetic photons generated and attenuated by probabilistic processes. By reducing the radiation dose to the patient, and thus the number of X-rays emitted from the tube, one effectively increases the statistical uncertainty in the gray value of each pixel in the image. That results in what is known as quantum mottle and is manifest as a grainy, noisy image. Patient radiation dose can be minimised by selecting a tube potential that produces the most-appropriate beam quality. That gives a good balance of attenuation by tissues (resulting in acceptable levels of contrast) while simultaneously permitting a sufficient quantity of X-ray photons to interact with the film and adequately expose an image that is relatively free of noise.

Mitigating image noise can be a considerable task, however. Resolving small structures can be difficult when their appearance is similar in amplitude to background noise (usually described by contrast-to-noise ratio). Statistical noise, known as quantum mottle, is the main contributor to noise in the image chain (4). If Quantum mottle approximately obeys a Poisson distribution, relating to the fact that it corresponds to the standard deviation in the number of photons that are attenuated or transmitted to produce signal in the image receptor, each X-ray directed at the patient can be considered a “sample” and increasing the sample size (or exposure from the tube) will reduce image noise accordingly. The relationship between standard deviation and sample size in a Poisson distribution is given as
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\[ \sigma \propto \frac{1}{\sqrt{n}} \]  

(1-1)

Where \( \sigma \) is the standard deviation in measurement values and \( n \) is the sample size. In order to reduce the standard deviation (or image noise) by one half, radiation dose to the patient would require a four-fold increase (4). In this way, patient dose, image noise, and contrast resolution are all inter-related. One can examine the issue in greater depth and consider that increasing the radiation output from the X-ray tube also involves the generation of excessive heat, which can shorten tube lifespan, and requires longer exposures which are more susceptible to patient motion artefacts (6).

All of these factors were given consideration over the course of this research. Improvements in image contrast (by the introduction of highly attenuating foreign media) allow for sacrifices in some other areas of image quality which may help reduce patient radiation dose, improve equipment life, reduce scan times, etc.

1.2 Interactions between Radiation and Matter

A contrast medium is a foreign material that may be administered into a patient to improve a radiographer’s ability to resolve anatomical regions of interest. The majority of soft tissue in the body, including most organs, muscle, and areas of vasculature induce very similar behaviour to incident X-rays; varying by only a few percent in terms of total attenuation relative to each other over the diagnostic X-ray energy range (9). To understand the limitation, and subsequently how to overcome it, one must have a clear understanding of the physical processes of attenuation and how to manipulate them for a given target volume.

Attenuation is the term applied to the combined absorption and scattering of X-ray photons as they are incident upon a material. Attenuation is comprised of three energy-dependent processes and as such, the probability of photon attenuation varies with changes in
1. Introduction

X-ray wavelength. These processes of attenuation include photoelectric absorption, coherent scattering (Rayleigh scattering) and incoherent scattering (Compton scattering) (6). The overall attenuation for a material at a given energy is given by its linear attenuation coefficient ($\mu$) which is the additive combination of $\mu$ values for each of these three processes (10):

$$\mu = \mu_R + \mu_C + \mu_{pe} \quad (1-2)$$

where $\mu$ is the combined linear attenuation coefficient, $\mu_R$ is the attenuation coefficient for Rayleigh scattering, $\mu_C$ is the attenuation coefficient of Compton scattering, and $\mu_{pe}$ is the attenuation coefficient for photoelectric effect.

Linear attenuation coefficient $\mu$ (given in the units cm$^{-1}$) or mass attenuation coefficient $\mu/\rho$ (cm$^2$/g), are the common means of quantifying a material’s ability to absorb and scatter radiation. In the body of this work, these terms are referred to on a multitude of occasions as they allow one to calculate how a contrast agent will affect the transmission of incident radiation through a patient. Moreover, they allow one to estimate how a layer of added beam filtration will shift the energy spectrum emitted from the tube.
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Figure 1.2: Plot of mass attenuation coefficient versus energy for each component process (Rayleigh scattering, Compton scattering, and photoelectric absorption) for the element Calcium (9).

In the case of incoherent, or Compton, scattering, the probability of attenuation is very low for photons below 10 keV in energy. Above that energy, attenuation coefficient remains relatively constant, as shown for calcium in Figure 1.2. This is roughly the case, regardless of material type (6). In the process of Compton scattering, incident X-rays interact with loosely-bound electrons in an atom’s outer shell. The photon is deflected from its course donating some energy to liberate and accelerate the now-unbound electron. The Compton process produces a secondary, scattered photon of lower energy and a scattered electron from the ionisation event (4). Rayleigh scattering (also known as coherent or classical scattering) involves an incident photon interacting with the electrons in an atom as a group. The process results in a negligible loss of the photon’s electromagnetic energy and the scattered secondary photon is projected in a nearly-forward direction. Rayleigh scattering is the least important of
1. Introduction

the attenuating events in diagnostic imaging because its probability very low for most materials when considering photons above 10 keV (also illustrated in Figure 1.2) (5).

Alternatively, the most important attenuation process in radiology is photoelectric absorption. Photoelectric absorption is caused when an incident X-ray loses all of its energy by ejecting one of an atom’s inner shell electrons. The ejected electron will have a kinetic energy equal to the difference in the incident photon energy and the binding energy of its atomic orbital (6):

\[ E_K = h\nu - E_B \]  

(1-3)

Where \( E_K \) is the kinetic energy of the ejected photoelectron, \( h\nu \) is the energy of the incident photon derived from Planck’s law, and \( E_B \) is the binding energy of the electron’s orbital. This process relies on a resonance between photon wavelength and the energy of the atom’s particular binding orbitals. Photons with more energy than the electron’s binding orbital (in keV) are available to be absorbed photoelectrically, while those with insufficient energy cannot. The binding energy of K- or L-shell electrons varies considerably between elements, thus the associated K- or L-edges varies accordingly (see Figure 1.9).

Not only do heavy elements have greater electron binding energies, they also contain a greater quantity of electrons (or higher electron density). Owing to those properties, it can be said that the photoelectric mass attenuation coefficient varies in approximately in proportion to \( Z^3 \) (6); that is, heavier elements have a much higher probability of absorbing X-rays than lighter ones. It is also a fair approximation to state that the probability of photoelectric absorption decreases with increasing photon energy (approximately \( \mu e \propto E^{-3} \)) (6). There is an important caveat to these statements. In order for a photon to eject a bound electron, its energy must exceed the binding energy of that electron’s orbital. Otherwise no absorption will occur. That means that in some cases, higher energy X-rays will be more likely to be absorbed because they have the ability to eject the
1. Introduction

inner K- or L-shell electrons of a particular element (known as the K-edge). Likewise, in some cases a photon will have a higher probability of absorption by a lighter element because of the particular binding energy associated with that atom’s K-edge.

![Mass attenuation coefficient of iodine for varying X-ray photon energy (in MeV).](image)

Note the declining trend with sharp jumps at 0.005 and 0.033 MeV (5 and 33 keV) corresponding to the L- and K-edges of the element, respectively.

From these attenuation processes, the probability of a photon interacting with a material or being transmitted unaltered depends on the type of material as well as an X-ray’s associated electromagnetic energy as described by its frequency, wavelength, or more commonly simply its equivalent electron voltage. From the above explanation it should be apparent that, in general, photons with higher energy (higher frequency, shorter wavelength) have a greater probability of transmission than their low-energy counterparts. The exception
occurs at certain energy thresholds that correspond to an atom’s electron binding energies. When the photon energy surpasses the binding energy at iodine’s K-shell at 33.2 keV, there is a distinctive increase in the probability of photon absorption by photoelectric effect (see Figure 1.3). While all elements contain a K-shell orbital, it’s only in heavy elements with many electrons that the binding energy of this orbital is sufficiently high to produce an edge in the diagnostic X-ray energy range (>10 keV). In the case of iodine, the K-edge occurs at 33.2 keV. In order to take advantage or iodine’s relatively high level of photo-electric absorption, radiographers must designate a tube potential that produces a large number of photons above that 33 keV threshold. For that reason (and also due to the size of the anatomical regions being examined), radiographs looking to identify the presence of iodinated contrast media are generally performed in the range of 70-140 kVp (effectively with photons between 40 and 100 keV). At the moment, iodinated CM is the clinical standard for examinations requiring intravenous media. Current imaging procedures have been optimised for the element. It has been the most widely used for roughly half a century, but in the first half century of radiology many different materials would be considered.

1.3 Radiographic Image Contrast

The collective term “image contrast” refers to the variations in pixel intensity (darkness, gray value, HU, etc) that occurs between two (generally adjacent) regions. Image contrast that is diagnostically valuable represents variation in X-ray attenuation by tissues that differ in thickness, density, or elemental composition. Here we will offer a brief explanation of subject contrast in radiography and how it relates to these properties of subject composition.

Subject contrast can be defined numerically as the difference in observed lightness/darkness in a region relative to the background. According to Wolbarst, contrast is defined as:
1. Introduction

\[ C = \frac{(L_{obj} - L_{bg})}{L_{bg}} \]  

(1-4)

where \( C \) is the subject contrast, \( L_{obj} \) is the lightness of the object region on the image, and \( L_{bg} \) is the lightness of the background (4). In digital radiographic systems, there is a linear response between the intensity of X-rays that interact with the image receptor and the brightness of the recorded image. In this case, contrast can be simplified as

\[ C = \frac{(I_{obj} - I_{bg})}{I_{bg}} \]  

(1-5)

where \( I_{obj} \) is the intensity of X-rays transmitted through the object region on the image, and \( I_{bg} \) is the intensity of X-rays transmitted through the background (or region of greatest intensity).

Figure 1.4: Relative transmission of X-rays and associated contrast values for regions of (A) the soft tissue with the same thickness, (B) the soft tissue with different thicknesses, (C) soft tissue with different densities, and (D) materials of different type and density (soft tissue and compact bone)
1. Introduction

<table>
<thead>
<tr>
<th>Material</th>
<th>Thickness (cm)</th>
<th>Density g/cm³</th>
<th>Mass Attenuation Coeff. ($\mu/\rho$) (cm²/g)</th>
<th>$I/I_o$</th>
<th>Contrast relative to background (3 cm soft tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft Tissue</td>
<td>3.00</td>
<td>1.00</td>
<td>0.23</td>
<td>0.51</td>
<td>0.00</td>
</tr>
<tr>
<td>Soft Tissue</td>
<td>2.00</td>
<td>1.00</td>
<td>0.23</td>
<td>0.64</td>
<td>0.25</td>
</tr>
<tr>
<td>Soft Tissue</td>
<td>3.00</td>
<td>0.50</td>
<td>0.23</td>
<td>0.71</td>
<td>0.40</td>
</tr>
<tr>
<td>Bone</td>
<td>3.00</td>
<td>1.85</td>
<td>0.42</td>
<td>0.09</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Table 1.1: Table of linear attenuation coefficients and transmitted intensities for 50 keV X-rays. The effect of changing material type, thickness, and density is shown by fluctuations in fraction of transmitted photons ($I/I_o$). Corresponding diagram is shown in Figure 1.4.

Attenuation by photons can be calculated according to the Beer-Lambert equation of exponential attenuation:

\[ I = I_0 e^{-(\mu/\rho)x} \]  

(1-6)

where $I$ is the transmitted X-ray intensity, $I_0$ is the incident X-ray intensity, ($\mu/\rho$) is the mass attenuation coefficient of a material at a given energy, $\rho$ is the material’s density, and $x$ is the photon path length. As discussed above, the probability of X-rays interacting with a material depends on photon energy and material type (5). Thus the value of ($\mu/\rho$), the mass attenuation coefficient, is a function of $E$ (photon energy, in keV) and $Z$ (attenuating material atomic #): ($\mu/\rho$)(E,Z). It is important to note that true exponential attenuation of X-rays only applies to mono-energetic photons and only when the influence of scattered, secondary X-rays is ignored (4).

The effect of different materials (type, density, or thickness) on image contrast is illustrated in Table 1.1 and Figure 1.4. Table 1.1 shows the calculated attenuation of a monoenergetic 50 keV beam as it passes through materials with different path length, or density. X-ray transmission depends partly on the amount of matter, or number of atoms, that the beam must cross. Thus reducing either density or path length allows incident X-rays to pass more freely (Figure 1.4 b & c). In the example shown above, decreasing soft tissue...
1. Introduction

thickness from 3 to 2 cm increases the number of 50 keV photons transmitted from 51 to 64%. That corresponds with a contrast value of 0.25 as given by Wolbarst (4).

![Diagram](image)

Figure 1.5: Attenuation of incident X-rays by multiple materials involves the combination of attenuation coefficients and thicknesses for each material.

The anatomy of a patient is rarely made up of a single material. Instead, each photon will encounter what can be considered layers of different materials; each with their own density and attenuation coefficients. Attenuation in this case occurs as the product of the fractional attenuation in each layer. Adding each layer to the Beer-Lambert equation and combining, it is given that:

\[
I = I_0 e^{-\left(\mu_1 x_1 + \mu_2 x_2 + \mu_3 x_3\right)}
\]

(1-7)

where \(\mu_n\) is the linear attenuation coefficient for material \(n\) and \(x_n\) is the thickness of material \(n\). Introducing a contrast agent to the body imparts atoms with a large attenuation coefficient without affecting target size. Optimally, these materials will selectively accumulate in a region of interest where they will decrease X-ray transmission and produce a contrasting
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shadow on the radiographic image; that shadow is often sufficient to allow delineation of otherwise unseen anatomy.

In a clinical setting, where the X-ray beam contains a broad spectrum of energies, accurately calculating the transmitted intensity is more complicated. The intensity of the beam must be considered at each discriminate photon energy level. That intensity varies according to the spectral energy distribution given by an X-ray tube’s potential, voltage waveform, the presence of filtration, target material, and target angle (6). Examples of X-ray spectra are shown in Figure 1.1. For a set of tube conditions (peak kilovoltage), the intensity of photons is a function of photon energy or wavelength:

\[ I_{OE} = I(kVp, E) \]  

(1-8)

where \( I_{OE} \) is the intensity of photons at a given energy level, \( I(kVp,E) \) is the function of intensity of X-ray energy for a tube potential of \( kVp \) peak kilovolts and at a photon energy level of \( E \) kilo-electron volts.

The overall attenuation or transmission of a diagnostic X-ray beam involves the integration of attenuation of all incident photon energies. Here we can incorporate equations combining materials and X-ray energy levels into:

\[ I = \int_{0}^{kVp} I_{OE}(kVp, E) \times e^{-\sum_{n}^{\infty} (\mu/\rho)(E,Z)_{n} \rho_{n} \times \rho_{n} + \ldots + (\mu/\rho)(E,Z)_{0} \rho_{0} \times \rho_{0}} dE \]  

(1-9)

where the functions of incident intensity \( I_{OE} = I(kVp, E) \) and \( (\mu/\rho)(E,Z) \) are described above. For each energy level, \( E \), all materials (1 to \( n \)) on an X-ray’s path are considered along with their associated densities and thicknesses. Incident intensity is integrated over all energies ranging from 0 to the peak kilovoltage, \( kVp \), in kilo-electron volts.

Again, such an equation does not account for the effects of scattered radiation. When a radiographic exposure occurs, some X-rays are absorbed in the body by photoelectric effect while others are scattered by Compton effect, producing secondary photons. In both cases,
the X-ray has been attenuated from its path. In the case of scattering, however, the secondary photon may still interact with the image receptor, albeit in a different location. Scatter radiation is produced by stochastic processes and its angle of deflection is random in nature (4). That means that it does not carry information and consequently its exposure on the image receptor does not act as a valuable representation of patient anatomy. In practice, scatter radiation is produced from many different positions within the body and it travels in a variety of directions. The contribution of millions of random, scattered photons results in a nearly-uniform “fogging” of the X-ray image (6).

Figure 1.6: Diagram of scatter radiation influencing radiographic contrast. Secondary X-rays produce a uniform fog, or exposure, over all regions of the image

Scatter radiation causes a decrease in subject contrast. It exposes areas representing radiolucent tissue just as it exposes areas of radiopaque tissue. Although it does not affect the difference in intensity between adjacent tissues, it does reduce the visible appearance of light and dark areas. Using the radiographic contrast equation \( C = (I_{obj} - I_{bg}) / I_{bg} \), it can be
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shown that increasing the exposure to two regions has no effect on $\Delta I$, $(I_{\text{obj}} - I_{\text{bg}})$, but it increases the value of $I_{\text{bg}}$ in the denominator. Let $s$ be the increase in radiation intensity at the image receptor due to uniform scatter radiation:

$$C = \frac{(I_{\text{obj}} + s) - (I_{\text{bg}} + s)}{(I_{\text{bg}} + s)} = \frac{I_{\text{obj}} - I_{\text{bg}}}{I_{\text{bg}} + s} = \frac{\Delta I}{I_{\text{bg}} + s}$$  \hspace{1cm} (1-10)

The addition of scatter radiation, or film fog, causes a reduction in the fractional contrast value. Because scatter radiation is random in nature, it may also add to statistical noise, or quantum mottle.

1.3.1 Image Contrast in Digital Systems

Digital imaging systems allow radiologists to artificially enhance contrast through post processing. This can involve real-time adjustments of image brightness (levelling) or contrast (windowing) to suit the desired appearance of a region-of-interest. Such adjustments invalidate Wolbarst’s contrast equation as a description of image quality. Increasing or decreasing the brightness across the entire image has the same effect on calculated contrast as increasing or decreasing the appearance of film fog. Let $k$ represent a uniform increase or decrease in image brightness due to levelling. If we incorporate this value into the contrast equation:

$$C = \frac{(I_{\text{obj}} + k) - (I_{\text{bg}} + k)}{(I_{\text{bg}} + k)} = \frac{I_{\text{obj}} - I_{\text{bg}}}{I_{\text{bg}} + k} = \frac{\Delta I}{I_{\text{bg}} + k}$$  \hspace{1cm} (1-11)

That suggests that adjusting the levelling can improve or degrade image quality regardless of the fact that there has been no physical change in how the image was captured, nor the amount of information it contains. Likewise, an adjustment to the windowing –via shifting the look-up-table to provide high contrast– does not really improve the information output of an image. Instead, such increases in contrast are augmented by a general decrease in image
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quality in some other respect. This is characterised by an equivalent increase in the amplitude image noise (4).

With digital imaging systems, it is conventional to use the term signal-to-noise ratio (SNR) or contrast-to-noise ratio (CNR), the term chosen for this work, as a descriptor of image contrast (4) (11) (12) (13). Contrast-to-noise ratio is given as a ratio of the average difference in signal between a region of interest and background ($\Delta I$) divided by the corresponding amplitude of stochastic noise in the image ($\sigma_{bg}$) (4). That is,

$$\text{CNR} = \frac{(I_{obj} - I_{bg})}{\sigma_{bg}}$$

where $\text{CNR}$ is the contrast-to-noise ratio, $I_{obj}$ is the intensity of exposure in the object region, $I_{bg}$ is the intensity in the background region, and $\sigma_{bg}$ is the standard deviation in pixel intensity for an otherwise uniform background region. In this way, post-processing has no effect on the quantitative description of image contrast. Uniformly increasing the intensity in both object and background regions (levelling) has no effect on either $\Delta I$ or $\sigma_{bg}$. Although increasing image contrast (windowing) increases the value of $\Delta I$, it also increases the amplitude of stochastic noise, $\sigma_{bg}$, by an equal degree. Thus the ratio of contrast divided by noise remains unchanged. It is appropriate because the image contains no more or less information.

Contrast measurements made in these experiments are quantified for best comparison using CNR values. Wherever possible, all experimental materials are compared within the same images, in order to reduce any bias that could result from fluctuations in image noise between exposures. When such methodology is not permitted, exposures are repeated with the same tube settings (kVp, mAs, distance, etc) for appropriate comparison.

1.3.2 Subject and Image contrast

It is worth making a distinction between what are known as subject and image contrast. Though the two are interrelated, the former refers to the special pattern of photons
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transmitted through the patient, or subject, prior to registration in the image receptor. Subject contrast may also be known as the primary image and it is representative solely of the differential attenuation of the X-ray beam (4). Image contrast refers to the recorded appearance of X-ray energy that has been deposited in the detector. It is worth making this distinction because processing in the imaging chain can cause some degradation in image quality (4). More importantly, some X-rays will pass through the image receptor undetected. This is particularly the case when using high-energy photons for diagnostic radiology. Imaging procedures that utilise a high kVp, employ highly penetrating X-rays to sufficiently transmit through the patient for certain procedures. These X-rays are also likely to penetrate a larger thickness of image receptor material before the majority are absorbed. Imaging procedures that use high kVp settings must utilise thicker layers of detector material to appropriately attenuate and record the primary image.

1.4 Contrast Media in Radiology

Since the advent of radiographic imaging, physicians have sought to artificially enhance the appearance of certain anatomical structures. From the outset of the era of "Roentgenograms", it was apparent that, while dense structures such as bone were clearly visualised, areas of muscle and soft tissue have similar appearance in the recorded image. Just one year after Roentgen was credited with the discovery of the X-ray, the first contrast agents were injected into the arterial system of cadavers (14) (15). The first contrast agents were used in pyelography, taking advantage of the relatively simple excretion of potentially dangerous heavy metals following examination. Wulff used a radiopaque suspension of bismuth subnitrate to image the bladder in 1904 (16). These experiments were quickly followed by trials of contrast media using a form of colloidal silver, Collargol, which was first employed in 1905 by Voelcker and Lichtenberg (17). Collargol produced high-quality images, allowing for the first time the diagnosis of renal pathologies and tumours. In first
several years of use limited side-effects were reported (18), but by 1912 several deaths had been linked to the material and its use in pyelography was widely rejected (19) (20) as discussed in (21). The next successful candidate for use as CM in pyelography was sodium iodide (22). This would also be the first implementation of iodinated contrast media, the basis for the most common set of contrast agents currently used in radiology.

Contrast-aided pyelography was heavily-explored in the early years of diagnostic imaging, but the discovery of an agent that could be safely administered intravenously would take much longer (21). It would take over a decade after use on cadavers before the first contrast-aided angiograph could be performed on a live human. In that case, a fluoroscopic examination was performed following the administration of a bismuth colloid suspension. The technique produced good visualisation of the heart and lungs (23). Such a contrast agent was also reported to show good visualisation of the kidneys (24). In the decades that followed angiocardography would continue to evolve with utilisation of catheterisation by Werner Forssmann (25) (26). Development of new iodine- and thorium-based compounds would vastly improve patient tolerance for intravenous contrast-aided examinations (though for a discussion of thorium’s long-term side-effects see “History of Nanoparticle Contrast Media” p.33) (27) (28).

The urinary tract and blood stream weren’t the only candidates for enhancement by contrast media. Due to low density, the margins of pulmonary tissue are poorly resolved beside the gas-filled airways of the bronchial tree. As early as 1917, physicians were experimenting with the introduction of a foreign, highly-attenuating material into these airways that would facilitate the diagnosis of diffuse pulmonary lesions (29). In that case, an iodinated emulsion was trialled, but dismissed due to its high toxicity and poor tolerance to the sensitive bronchial tissues. Gases were also used as negative contrast agents in
arthrograms and neuroradiology (30) (31). Carbon dioxide gas remains in some use, primarily in for insufflation of the colon.

The gastro-intestinal (GI) tract presents fewer hurdles for the introduction of contrast media because its contents are not entirely internalised within the body. The first GI agents were evaluated shortly after the discovery of the X-ray. Successful images using bismuth subnitrate were reported as early as 1897 (32) (33). The use of bismuth subnitrate and an alternative compound, Bismuth subcarbonate, were eventually discontinued due to toxicity issues (34) (35). The material that would largely replace bismuth-based CM, barium sulphate, was developed for clinical use in 1910 (36).

In contrast-aided radiographic procedures, toxicity of contrast media would continue to be an issue for the next several decades. In fact, of the prospective contrast agents used in the first several decades of X-ray imaging, only the Gastrointestinal agent Barium Sulphate, remains in any clinical use (36). Modern contrast media represent a steady evolution of pharmaceutical technology and discovery through trial and error, but even today, some patients have difficulty tolerating radiopaque contrast media. Regardless of improvements in image quality, research into techniques which minimise the administered dosage of CM are always valuable.

1.4.1 Intravenous Contrast Media

Intravenous contrast agents are designed to be injected into the bloodstream where they will enhance the appearance of associated anatomical features. In these cases the materials are largely confined within the margins of epithelial cells that line the circulatory and excretory systems. Intravenous agents are given the name blood-pool agents (due to their region of enhancement) or extra-cellular fluid agents (ECF) because their distribution is confined to a volume outside of cell membranes. Depending on the chemical formulation,
blood pool agents may tend to linger in some areas over others. This can be related to the material’s viscosity, specific interactions with cellular proteins, or the presence of pathologies.

Intravenous CM is often used to visualise tumours. Due to high metabolic activity and rapid growth rate, tumours are characterised by markedly high levels of blood flow. During growth, the tumour structure rapidly develops new blood vessels which may be convoluted and “leaky” in nature. This rapid angiogenesis is exploited in contrast-aided radiography. Malignant tissue tends to accumulate more intravenous contrast media than healthy tissue. Depending on the region and type of tumour, intravenous CM may also have a longer retention time in these regions of unhealthy tissue. A series of radiographs captured at different time points can record the variation in enhancement prior to- and just following injection. These types of temporal images indicate a region’s relationship to the bloodstream. It may also indicate some aspects of the region’s function, by indicating the amount of time required to excrete the radiopaque CM. This can be valuable to differentiate between malignant tissue or otherwise-benign lesions, for example, in hepatic CT (37).

1.4.2 Iodinated Contrast Media

Iodinated contrast media have taken a number of different molecular forms in their history. Even modern incarnations vary slightly depending on the manufacturer, but they generally share similar benzene ring structures that offer remarkable stability, solubility, and low toxicity. Wolf remarks that unlike any other pharmaceutical, iodinated contrast agents are the only materials that can be injected at a rate of grams per second into patients (38). In many ways, that is a truly remarkable feat. Nonetheless, these materials are still associated with complications, and, although less than their predecessors, the dangers need be considered. A clinician would not, for example, inject a full angiographic dosage of iodinated
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CM into an otherwise healthy person unless it was deemed diagnostically valuable. Thus, improvements in toxicity are always welcome. Moreover, the effort to improve image quality and diagnostic specificity is also worthwhile. The history of iodinated contrast media, relating to its different molecular forms, reduction of toxicity, and changes in its applications to radiology are a narrative of scientific discovery. They provide lessons for those designing new species of contrast media and offer solutions to overcome some of the hurdles associated with opacifying structures and pathologies of interest in a radiograph.
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Figure 1.7: Molecular formulas for various iodinated CM compounds. Figures are arranged from earliest (top) to most-recent (bottom). Note the incorporation of a greater number of iodine atoms per molecule and addition of many hydroxyl groups to maintain solubility while
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... reducing hypertonicity/osmolality. Chemical formulas and models recreated from *(21) and †(39)

The first widespread use of iodinated intravenous CM was in urography. The radiographic procedures, known as pyelographs, involved the injection of contrast into the urinary tract via urethra. While the process was effective in terms of the images produced, it was also invasive and generally impractical as a long-term solution to imaging the excretory system (21). Instead, it was preferable to introduce the radiopaque material either orally or through injection. Iodinated salts and compounds were (and still are) rapidly excreted from the blood stream to the urinary tract via the kidneys. Such administration prior to imaging, enabled physicians to delineate the margins of renal tissue, ureters, and bladder for diagnosis of any associated conditions without the drawbacks of catheterisation required in retrograde pyelography. Early reports of enhancement in the urinary tract from excretion of iodine were seen in syphilis patients prescribed sodium iodide as part of their treatment regime (40). Image contrast was insufficient, in this case, to be diagnostically valuable, but it led to further research in excretory use of iodinated CM. In 1929, Swick and von Lichtenberg used an iodinated compound purposely designed for solubility and tolerance to record a successful urogram following intravenous injection (41) (21).

Early iodinated contrast media was based on sodium iodide and potassium iodide salts. These were highly soluble, but only the sodium iodide species displayed an acceptable level of toxicity in early trials (22) (42). Neither material was sufficiently tolerable for intravenous administration. Swick and his collaborators experimented with iodine bound to benzene rings. They developed the first widely-used intravenous iodinated CM, Uroselectan (see Figure 1.7). Two years later, di-iodinated variants containing 2 iodine atoms per molecule led to further improvements in solubility and toxicity (21). Tri-iodinated compounds such as diatrizoate would follow (43).
The addition of more iodine atoms was not resolving all of the side-effects related to iodinated CM. In 1969, Torsten Almén suggested that the high osmolarity of the materials in use was largely contributing to toxicity (44). These materials were hypertonic compared to normal blood plasma, which resulted in an unnaturally large gradient to pull ions across the membranes of cells or through glomerular tissue in the kidneys. Older incarnations of contrast media were given the distinction High Osmolar Contrast Media (HOCM). By removing the salt-forming carboxyl groups (which required a balance of free sodium ions) and adding hydroxyl groups to maintain solubility, Almén was able to produce a molecule with much lower osmolarity than previous iodinated species. The compounds became known as Low Osmolar Contrast Media (LOCM) (see Iohexol Figure 1.7). He also suggested that synthesis of nearly-spherical molecules would lower the viscosity of these highly-concentrated solutions. That, in turn, improved tolerability and increased injection rate.

The latest iodinated contrast agents are known as dimeric, iso-osmolar, non-ionic CM. This distinction incorporates lessons from each step of the approximately 75 year evolution of iodinated compounds. These compounds are dimeric, meaning they incorporate two nearly-identical copies of tri-iodinated benzene ring monomers to maximise the number of iodine atoms per molecule (6 in total). The large in size to reduces osmolarity because radiopaque atoms are incorporated into fewer molecules in the solution’s volume. In the case of iso-osmolar CM, the osmolarity of the injected CM is comparable to that of blood plasma and thus has minimal affect on the tonicity of the fluid space. It is non-ionic, which likewise improves osmolarity and reduces pain upon injection (45). The structure of a modern iodinated CM compound, iodixanol (commercially known as “Visipaque”), is shown in Figure 1.7.
1.4.3 Adverse Effects of Iodinated CM

When describing the effects of a contrast media, there is a separation between those classified as primary and secondary. It can be said that, the primary effect of an iodinated contrast agent is solely the attenuation of incident radiation. Any other secondary effect is undesirable and falls into the category of an adverse effect or event. In most patients, such effects related to iodinated CM are relatively minor and may not even manifest in a detectible manner (46). In some patients however, there is a notable correlation between the administration of iodinated contrast media and decreases in renal function and even renal failure (47). It is still, however, important to be aware of any impacts on body function that could elicit a negative reaction.

There has been significant exploration into the secondary effects of iodinated contrast media (48-50). Historically adverse events were much more common and severe than they are today. Improvements can be attributed to the use of non-ionic, tri-iodinated compounds with low osmolality (51, 52). Adverse events can be considered dose-dependent. They are most commonly seen in procedures that require large volumes of contrast media such as arteriography (45). In this way, efforts to improve image quality and thereby reduce the quantity of iodinated CM are valuable for reducing the frequency of adverse events.

The mechanism of renal malfunction following administration of iodinated CM is often related to the solution’s hypertonicity and viscosity (47). After injection of HOCM, and to some degree LOCM, contrast medium molecules equilibrate within the intravascular compartment. These solutions have higher relative osmolality than human plasma which induces across capillary membranes into the vascular space (47). The high solute concentration, hypertonicity, also creates a strong gradient for the flow of CM particles across the glomerular membrane of into the renal tubules (53). Normal kidney function is marked by the filtration and subsequent reabsorption of water by renal tissue. Contrast molecules that
collect in the excretory volume of the kidneys continue to exert and osmotic force which reduces the reabsorption of water in these tissues (47). Such effects are marked by a decrease in glomerular filtration rate (GFR) in patients following contrast-aided radiography (54).

Most individuals tolerate the effects of intravenous iodinated CM without complication. There is a cohort of patients who have been identified as predisposed to adverse events. Side effects have been correlated to several risk factors which, particularly when combined, lead to greater likelihood of renal failure (55). These include pre-existing renal insufficiency, diabetes mellitus, dehydration, cardiovascular disease, concurrent use of diuretics, age above 70 years, myeloma, hypertension, and hyperuricemia (55-57). Renal insufficiency may be ascertained by a low GFR (tested by measuring the patient’s serum creatinine levels) (51). In patients presenting with these distinguishing characteristics, it is recommended that precautions be taken prior to radiocontrast examination. These include hydration (either orally or intravenously), use of LOCM or iso-osmolar variant of CM, discontinuing use of nephrotoxic drugs for a minimum of 24 hours, and consideration of alternate imaging techniques (58).

1.4.4 Current Trends

Issues correlating to kidney-related adverse events have been heavily examined over recent decades. Following the advent of non-ionic iso-osmolar contrast media, however, progress has been very limited (59). In such a complicated system as the human body, the mechanisms that produce either beneficial or detrimental effects are not easily understood. Compounding the issue, treatments that show promising results in vitro may not translate to a tangible benefit when utilised in patients (60). For example, although an experimental contrast agent may display a good safety profile in cultured nephron epithelial cells, that says little about its safety in vivo. In reality, the cells are not simply in an environment with a
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concentrated foreign molecule, their function clearing the material across boundaries of the renal medulla must be tested (61). There are signalling pathways between neighbouring tissues to be considered (62). So in fact, there can be hundreds of seemingly-hidden factors that may come into play.

A review of recent research shows considerable efforts in production of nanoparticle-based contrast media (63-68). The rationale is that the inclusion of hundreds or thousands of radiopaque atoms on a single molecule –or, more appropriately, particle- can reduce the inherent stress on renal tissue during clearance. Such an approach has been applied, not just with gold, but other heavy metals and, of note, iodine itself (63). These are not the only current trends in radiographic contrast media, however.

One approach to mitigating contrast-induce adverse events is to use a combination of different contrast media. The implication in this method is that each individual CM is more or less safe until it is the administered dosage exceeds a certain threshold and, more importantly, that the toxic effect of each CM is different. That is, the negative effects of the agents will not compound each other. One study evaluated a combination of gadolinium and iodinated CM for use in angiography (69). There is still debate regarding the nephrotoxicity of gadolinium-based contrast agents (70-72). Gadolinium contrast agents have shorter clearance half lives than iodinated CM (the half lives of iopamidol and gadodiamide are 70 and 34 hours, respectively (73) (74). That suggests that they are safer in patients with low tubuloglomerular filtration rates, but recent research suggests that pathological effects using gadolinium-based CM are actually more severe and toxicity in vitro is greater. In Badiola’s study, the combination of contrast agents also showed limited contrast enhancement given the mass of radiopaque material administered (40 cc volume of 4 parts magnevist to 1 part optiray). Images were collected in dual-energy subtraction angiography (DSA) at potentials of 68 and 90 kVp.
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Improving the resolution of contrast media is a constant process that reaps tangible benefits in terms of a reduction in either patient radiation dose or administered contrast volume. Prior to consideration for widespread usage in mammography, Arvanitis and Speller proposed a methodology for maximising iodine detection at low diagnostic tube potentials. Using a similar protocol as those proposed in the latter portions of this research (dual energy subtraction with selective filter materials, see Chapter 7), they were able to achieve a relatively high signal-to-noise ratio of 5 in a ROI containing iodine with low CM concentration (\(< 3 \text{ mg I cm}^{-2}\)) and with 10% of the absorbed dose compared to current clinical mammography protocols (75).

1.5 Nanotechnology

1.5.1 Properties of Nanoparticles

Nanoparticles are characterised as cluster-based molecules with dimensions between 1 and 100 nanometres. The exact limits of this classification are, in a way, arbitrary. There would be, for example, very little physiochemical difference between a metallic particle that was 90 nm compared to one that was 110 nm. But the distinction of being a material in the sub-100 nm size range serves to indicate that the cluster should be considered as neither a group of single atoms nor as an infinitely large solid lattice (76).

When particles reach the extremes of miniaturisation, below roughly 10 nanometres, the normal bonding characteristics of nanoparticle atoms cease to resemble those of conventional, bulk solids (76). In some instances bond distances will decrease due to tension imparted by a large percentage of surface atoms (77). In other cases, the crystal structure may change form altogether to produce nanotubes, fullerenes, or compact clusters (76). In such cases, the behaviour of electrons in the structure (particularly those that correspond to molecular, bonding orbitals) is unique to these miniature nanoparticles. This can donate novel
properties in terms of catalysis or semi-conduction (78, 79). Worth consideration in designing radiopaque particles, nanostructures are often characterised by their specific absorption of soft X-ray photons in X-ray absorption spectroscopy (XAS) (80, 81).

In order to be viable as a radiographic contrast agent, a material must have four characteristics. It has to contain a high density of radiopaque atoms (generally those with an atomic mass number greater than 50). It also has to be smaller than the narrowest capillaries in the human blood stream. This commonly limits dimensions to a maximum of roughly 7 µm (82). In practice, much smaller dimensions are preferential, but most synthesis procedures for nanoparticles or even so-called microparticles fit easily within such size restrictions. Contrast solutions are preferably aqueous in nature, meaning the particles are stable in a polar solvent such as water or phosphate-buffered saline (PBS). The capping layer (and preferentially, the metallic/radiopaque core) needs to be non-toxic and allow safe clearance from the bloodstream. This feat can be somewhat difficult since the capping agent is also responsible for preventing the irreversible aggregation of nanoparticles. To use these materials as a traditional, blood-pool agent (injected at high-concentration) is much more difficult than if particles can be successfully targeted to tissues of interest where they would remain for a period of hours. Conjugated nanoparticles may also incorporate a monoclonal antibody onto the surface of the structure to improve targeting specificity to certain cell-types (83).

The radiopaque core of a prospective nanoparticle CM will contain anywhere between less than a dozen to over one-hundred thousand heavy atoms. The most common means of producing the core structure is through chemical synthesis, though physical processes such as laser ablation are viable as well (84, 85). Synthesis protocols have been described for the production of several different elements that might be suitable for radiographic applications. The reduction of bismuth chloride (BiCl₃) with tert-butyl alcohol (t-BuONa) activated sodium hydride can produce gram-scale quantities of nearly monodisperse particles with a weak
alkoxide surface ligand that can be easily modified (86). Galperin utilised 30 nm iodinated nanoparticles designed as polymers of MAOETIB (triiodophenyl methacrylate, 2-methacryloyloxyethyl(2,3,5-triiodobenzoate)) monomers. These particles were similar in nature to dimeric iodinated CM (see Figure 1.7), but containing several hundred copies of the triiodinated monomer groups (63). A detailed description of synthesis procedures for gold nanoparticles trialled as CM appears in section 1.6.2.
1.6 Gold Nanoparticles as contrast media

Figure 1.8: Radiograph of a hand phantom displaying highly radiopaque gold ring

1.6.1 History of Nanoparticle Contrast Media

The use of nanoparticulate contrast media is not an entirely new trend. Advances in chemical synthesis, improved understanding of biochemical pathways, and improvements in characterising such small particles have led to a rapid growth in nanotechnology of late, but the origins of nanotechnology in radiography go back much further. The earliest mention of nanoparticle contrast media was the use of colloidal silver for imaging the urinary tract (17).
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While the notion of the utilising “nanoparticles” per-se was surely irrelevant at the time, colloidal suspensions offered a means of putting heavy metals into a liquid substrate.

Over the following years, several different metallic nanoparticle CMs would be considered, some even widely used. Bismuth suspensions mentioned above (see page 19), were eventually dismissed due to their cost and high toxicity. Animal tests on intravenous use of colloidal tin (Stannic Oxide, SnO$_2$) were performed. The particles showed good radiopacity and were tolerated well, but the material was dismissed as a prospective CM due to its long-term body retention (87, 88).

The most widely used contrast agent in the early 20$^{th}$ century was a nanoparticulate species based on the heavy element (and natural radionuclide) thorium. Colloidal ThO$_2$ found many applications as a radiographic contrast agent. It was first used in pyelography and bronchography (29, 89). Use became highly widespread several years later after its demonstration as a viable intravenous agent with excellent performance in cerebral arteriography and in liver imaging (90, 91). Though never endorsed by the American Medical Association, Thorotrast (as the thorium-based CM was known) became the most widely-used contrast agent in between 1928 and 1950 where it was estimated that over 10 tons of the material was consumed; exposing millions of people to the element’s emitted alpha particles (92) (93). Although imaging performance was excellent and acute side-effects were minimal, nearly all of the injected thorium was retained within the body (94). Due to the element’s long half-life ($t_{1/2} = 1.41 \times 10^{10}$ yr), patients suffered from the effects of long-term radiation exposure. Many cases of malignancies and blood disorders were reported decades after the material had been administered (95).

The use of Thorotrast lingers as a cautionary tale to pharmacologists and radiologists. It shows the importance of designing contrast media that mitigates both short- and long-term side-effects. In particular, it highlights that these heavy metals must be sufficiently removed
from the body following injection. Though a material such as gold is highly inert and occurs as a stable isotope, the long-term effects on cells and tissues may take years to surface. Concerns over such occurrences are alleviated if there is minimal retention of the contrast agent.

1.6.2 Previous Work with Gold Nanoparticle CM

The first documented instance of a contrast-enhanced radiograph was a 1986 case report published by Marchello De Maria in Radiology (96). The patient, a 65 year old female, had been admitted with pain in her right upper quadrant and was evaluated for possible biliary stones. On CT scans, there were inhomogeneous areas of high attenuation in the hepatic parenchyma (up to 215 HU) that were inconsistent with parenchymal disease. Further examination of the patient’s history did not indicate cause for excess iron deposit in the liver, but she had undergone five years of colloidal gold therapy for rheumatoid arthritis in the years prior. Biopsy of the liver showed clusters of reticuloendothelial cells in the portobiliary spaces filled with a granular brownish material that were shown positively as gold. The paper suggested that radiologists confirm the case history of patients for previous use of gold colloids if they present with paradoxically dense hepatic tissue on CT.

The first major recent publication on gold nanoparticle contrast media was by Hainfeld et al. in 2006 (64). In the paper, a suspension of 1.9 nm nanoparticles was injected intravenously into mice which were subsequently imaged with mammographic equipment (22 kVp). Toxicity results from these experiments were relatively good. Mice injected with 2.7 g Au per kg body mass survived over one year without complications. The LD$_{50}$ of the material, however, was shown to be only slightly greater at 3.2 g Au kg$^{-1}$. Histological examination of excised tissues indicated that greatest gold accumulation was in the liver, with the mass increasing steadily over the first 10 hours post-injection. Gold was found in renal
tissue to a similar extent. Nanoparticles, even though untargeted, showed specificity for malignant tissue as well. Images showed exceptional enhancement of vasculature or renal structure and ureters depending on the time point post-injection.

Our research into gold nanoparticle CM initiated after the publication by Hainfeld et al. Although their research demonstrated that contrast enhancement could be obtained in vivo through the introduction of AuNPs, their results were characterised qualitatively and using small animals with very low energy X-rays. The outset of our research was aimed to supplement this initial publication by quantifying contrast enhancement using an image phantom containing AuNP contrast media. This was completed using radiographic techniques comparable to those for human thoracic imaging protocols at a variety of tube potentials (97) (submitted 31 July 2008 and published online 29 April 2009). For a detailed description of this work see Chapters 2 & 3.

A study by Cai et al. utilised poly-ethylene glycol-stabilised gold nanoparticles to image Balb/c mice with induced fibrosarcoma in computed tomography (98). 38 nm gold nanoparticles were synthesised by the Turkevich method (99) and modified by the addition of a PEG sulfhydryl capping group. Mice were evaluated in micro CT at 50 kVp following injection of AuNP-PEG contrast agent (2.5 µmol Au/g body weight) via tail vein with images recorded at time points over 3 days. Bloodpool enhancement up to 130 HU was shown and circulation half-life of approximately 15 hours. No renal excretion was evident. This was attributed to the relatively large particle diameter, 38 nm, impeding excretion by glomerular filtration in the kidneys. In vitro images of contrast media indicated 2.7 times greater enhancement by AuNP-PEG suspension than iodinated CM (Lobrix). Toxicity tests did not indicate any decrease in viability in vitro or in vivo and particles were not found to cross the blood brain barrier.
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A similar set of experiments were performed by Kim et al. with a different animal model and images acquired at higher X-ray energy (66). Poly-ethylene glycol-coated gold nanoparticles were synthesised by Turkevich technique and subsequent addition of PEG sulphydryl moiety. TEM images of particles showed nearly monodisperse size distribution with mean diameter of approximately 30 nm. Sprague-Sawley rats were prepared with N1S1 Hepatoma cells. CT images were acquired at 120 kVp on GE 64-slice CT scanner. *In vivo* images indicated increasing blood pool contrast enhancement over the first hour post-injection. Concentration in these regions (left ventricle, aortic arch, and inferior vena cava) gradually declined over the following 23 hours. Over that time period, HU enhancement of the spleen and liver subsequently increased indicating retention in hepatic tissue and accumulation in the spleen by AuNP phagocytosis by macrophage cells. *In vitro* CT images of AuNP suspension and Ultravist iodinated CM indicated 5.7 times greater attenuation coefficient of gold than iodine. No measurable cytotoxicity was found in *in vitro* MTT assay below concentrations of 1 mg Au per mL.

A more complex species of gold nanoparticle was evaluated for use as a CT contrast agent by Kojima et al. (67). Polyamidoamine (PAMAM) dendrimers have been described as a means of encapsulating materials for drug delivery (100). These act as template shells which can encapsulate foreign materials. In Kojima’s experiment, the PAMAM dendrimers were modified with biocompatible poly-ethylene glycol to improve cytotoxicity. 2 nm gold nanoparticle “seeds” were then grown inside the encapsulating dendrimer shell by the reduction of chloroaurate ions by ascorbic acid. The technique produced 4 batches of dendrimer-encapsulated nanoparticles with diameters of 3, 5, 7, and 8 nm. CT images were collected *in vivo* in a ddY mouse model following injection of gold nanoparticle contrast media (200 µL, 117 mg Au per mL) or iopamidol (200 µL, 150 mg I per mL). Images were acquired on an animal CT scanner at 210 kVp. AuNP-dendrimer contrast media showed peak
blood pool enhancement in the first minute after injection, with decreasing retention time until 10 minutes when no intravenous enhancement was apparent. The nanoparticles showed increasing accumulation in the liver and minimal renal excretion after 1 hour. The kinetics were distinctly different to iopamidol which showed nearly complete clearance through the first 20 minutes.

The promise of successfully targeting nanoparticles is only beginning to take shape. Heparin-coated AuNPs have been demonstrated as a successful agent to enhance hepatic lesions in CT imaging (101). The liver is a good candidate for targeted contrast media due to the frequency of hepatic metastases and the limited retention time of iodinated compounds in the region. In the study, heparin-coated nanoparticles showed displayed good stability in water and phosphate-buffered saline (PBS). Good visualisation of the liver and spleen was shown in CT images. The biodistribution of the conjugated particles was studied by single-photon-emission computed tomography (SPECT) using radiolabled heparing compounds. Findings showed over 50% of AuNPs accumulated in the liver and spleen.

Nanoparticles needn’t target malignant tissue to offer clinically relevant information. Eck et al. synthesised gold nanoparticles bound to a CD4 antibody to specifically target the lymphatic system (102). Gold nanoparticle cores (28 and 38 nm) were coated with a polyethylene glycol stabilising agent which were then outwardly bound to an anti-mouse CD4 monoclonal antibody. Mice were imaged with micro CT at 80 kVp. CD4-linked nanoparticles showed greater contrast enhancement than an IgG-bound gold nanoparticle control. Greatest enhancement was seen 48 hours post-injection, indicating that the nanostructures were selectively retained by cells of the lymph nodes. Authors suggested that macrophages, T-cells and other scavengers that bear the CD4 receptor collect the nanoparticle conjugates over a period of several hours, eventually transporting them to the lymph nodes where they increase local CT density. A full histological examination was not performed, however TEM of thin
slices of lymph node tissue revealed the presence of gold nanoparticle aggregates compartmentalised within the cellular spaces.

Another study aimed to harness the increased metabolic activity of tumour cells to improve nanoparticle specificity and subsequent image contrast in CT (103). 2-deoxy-D-glucose was bound to the surface of 4nm spherical gold nanoparticles. The suspensions of deoxyglucose-labelled and unlabelled gold nanoparticles were incubated in vitro with human alveolar epithelial cancer cell line, A-549 and imaged with micro-CT. Results showed approximately four times greater nanoparticle uptake for labelled nanoparticles compared to unlabelled species. Such a technique would combine the functional imaging capabilities of PET imaging with the improved resolution of X-ray CT imaging.

Similar in vitro experiments were performed with antibody-conjugated nanoparticles. Since these types of particles would discriminate between cells on the basis of over-expressed surface proteins, it is anticipated that with proper design they would offer greater specificity for malignant cells. A study by Popovtzer et al. produced similar results to those with deoxyglucose-labelled gold nanoparticles (104). Gold nanoparticles were labelled with UM-A9 antibodies, which show high specificity for squamous cell carcinoma, a common form of head an neck cancer (105). Popovtzer’s findings demonstrated 3-4 times higher HU values for correctly-targeted gold nanoparticles than cases where nanoparticles were targeted with a non-matching antibody. The targeted nanoparticles also demonstrated higher specificity for cancer cells than for non-malignant tissues.

Gold nanoparticles have also been proposed a dual-modality imaging agent; combining x-ray imaging with surface-enhanced Raman spectroscopy (SERS) (106). This technique, proposed by Ming Xiao et al., would combine the whole-body imaging capabilities of computed tomography with the high-resolution capabilities of optical imaging, albeit only at limited tissue depths. In the study, gold nanoparticles (20 – 120 nm) were
bound to one of six dye molecules. When bound to the nanoparticles, the dye materials exhibited characteristic optical Raman spectra indicative of the particular conjugated nanoparticle species. Good contrast enhancement was shown in CT imaging (up to 1000 HU at 12 mg Au/mL) in phantom. In vivo a strong Raman spectrum was shown even at lowest concentration (25 µg/mL). Greatest CT contrast enhancement was shown in the mouse spleen (432 HU) at 24 hours post-injection. Enhancement of other soft tissues was minimal at 100 HU. Histological examination of spleen tissue indicated that the nanoparticles had been internalised in local cells and preferentially accumulated in the endosomes.

A study by Alric et al. incorporated gadolinium chelates onto the surface of a thiol-derivitised gold nanoparticle core for dual-modality CT and MRI imaging as well as dose enhancement in microbeam radiotherapy (107). The conjugation of paramagnetic Gd$^{3+}$ species onto the particle structure created positive contrast enhancement on T$_1$-weighted MRI images. The particles were evaluated in vivo in a rat model with 9L gliosarcoma cell line. The imaging capabilities were measured in MRI and synchrotron radiation computed tomography (SRCT). Mono-energetic SRCT images were captured at 80.5 and 80.9 keV and subtracted to highlight the presence of gold atoms by K-edge effect. Greatest enhancement was shown in the kidneys, ureters and bladder indicating standard excretion route without undesirable nonspecific cellular accumulation. Only in rats bearing 9L gliosarcoma tumours, nanoparticles were found to cross the blood brain barrier; evidenced by moderate tumour enhancement in T$_1$-weighted MRI images. The dose-enhancement effects in microslit synchrotron radiotherapy were evaluated in terms of survival time. Mean survival time increased from 17.5 to 27.5 days between a control group and a group that received nanoparticle injection (1.5 mL, [Au] = 50.7 mM and [Gd] = 5 mM) combined with microbeam radiation. Cell viability studies using splenocytes and HeLa cells indicated
comparable toxicity between Gd conjugated gold nanoparticles, unconjugated gold nanoparticles, and a sucrose control.

Not all experiments investigating gold nanoparticles have focused on image quality in vivo or in vitro. A 2009 study by Noritaka Yusa et al. used Monte Carlo simulation to evaluate contrast enhancement of colloidal gold (108). In their study, a generalised tissue phantom was simulated containing a contrast region made of either bone or concentrated gold suspension (10, 1, 0.5, 0.2, & 0.1% w/v). The aim was to compare the appearance of gold against normal contrasting features in an X-ray image. Yusa adjusted the energy of the incident X-rays in his experiment to determine the significance of the Au K-edge to a diagnostic spectrum of X-rays. Beams of monochromatic X-rays were evaluated as well. The results have been reproduced in Table 1.2 & Table 1.3. In general, the appearance of bone was similar to gold at concentration of 1.0% weight to volume. The greatest enhancement of gold relative to osseous tissue was shown with monochromated X-rays at 88 keV, slightly above the 80.7 keV Au K-edge. Using polychromatic X-rays, as emitted from a vacuum tube, greatest Au enhancement relative to bone was shown at lowest energy: 60 kVp. The authors suggest, however, that acceptable levels of contrast enhancement can be obtained with a standard X-ray tube, and that improvement in visualisation reaped by implementing monoenergetic X-rays would not warrant the difficulty and expense involved.
### Table 1.2 Simulated image contrast due to X-rays from X-ray Tubes: I (0.5 cm) (108)

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<th>d (cm)</th>
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1. Introduction

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Table 1.3: Simulated Image contrast due to monochromatic X-rays: I (0.5 cm) (108)

1.6.3 X-ray attenuation by Au

Attributing to the element’s high atomic number, it is anticipated that gold will be more efficient at attenuating incident X-ray photons by photoelectric effect than conventional contrast media based on iodine, barium, or even gadolinium ($Z_{\text{Au}}=79$, $Z_{\text{I}}=53$, $Z_{\text{Ba}}=56$, $Z_{\text{Gd}}=64$) (6). Gold solid’s high density (19.3 g/cm³) permits very high linear attenuation coefficients in the energy range of the diagnostic X-rays. These characteristics indicate that gold can absorb a large quantity of incident photons while occupying a small volume within the extracellular space.

Due to the energy-dependence of the attenuation processes (in particular with heavy elements), a simplified description of a material’s attenuation relative to human tissue is
1. Introduction

difficult to achieve. That is, the generalisation that gold has a 600 times greater linear attenuation coefficient than soft tissue is only true in some instances (for example, at 50 keV and 100 keV). At 80 keV, slightly below the K-edge of Au, the element’s linear attenuation coefficient is only 230 times greater. So depending on the energy of X-rays involved, the relative attenuation by gold compared to other tissues in the body can vary 2-3 fold. The mass attenuation coefficients of gold and iodine are shown in Figure 1.9.

Figure 1.9: Tabulated mass attenuation coefficients of gold and iodine between 0 and 150 keV (up to 0.15 MeV). (9)
1. Introduction

1.7 Thesis Objectives

The goals of this thesis have been to improve the detection of gold nanoparticle-based contrast agents. This includes optimising the radiographic exposure parameters. This has been investigated by adjusting the energy range to selectively improve the contrast of gold atoms relative to the other elements that primarily compose the tissues of the body. Several aspects of radiologic image formation were to be considered including the addition of filtration material and modification of image receptors. The importance of gold particle size and structure in terms of contrast enhancement has received attention as well.

This project has integrated experiments with real-world images alongside analytical evaluations. Numerical analyses include exploration of tabulated attenuation processes and their relevance to detection of gold in diagnostic radiology. Monte Carlo simulations supplement those calculations. These simulations allow the investigation of a variety of exposure settings that might be impractical or overly costly for basic experimentation. These also allow the acquisition of data regarding the spectra of X-rays absorbed in the patient, contrast material, and those detected within the image receptor that are very difficult to obtain through measurement.

Many of these analytical investigations have been compared to empirical data from real-world phantom experiments to validate these directed hypotheses. Phantom studies have the advantage of being generalisable over a range of anatomical regions and image modalities. They are also less susceptible to statistical variation between image sets.

The aim of this work is to identify the ideal exposure factors for recording the presence of gold-based contrast media in radiography. Images have been simulated by Monte Carlo method over a broad range of tube potentials and with varying image receptor properties (fluorescence/scintillation material, coating weight). This technique offers several advantages from both practical and mathematical standpoints. Materials can be compared for contrast-to-
noise ratio in much the same way as real-world images, but simultaneously enables the extraction of other relevant data including patient dose and absorbed/transmitted energy spectra at a variety of positions in the X-rays path. Although the focus of this research applied to the attenuating properties of gold, similar techniques can be employed for optimisation of other elements (bismuth, gadolinium, iodine etc).

Chapters in this thesis have been written as a stand-alone summary of each experiment. Each chapter contains its own brief introduction, description of methods, results, discussion and concluding statements. For ease of reading, it has been commented that it may help to look through each chapter’s figures before reading the sections sequentially.
2 Projection and Computed Tomographic Imaging

2.1 Introduction

X-ray images may be recorded using one of two geometric techniques that we will classify as either projection-type imaging or computed tomography (CT). In projection imaging, a divergent beam of X-rays is emitted by a stationary X-ray tube. In this case, the X-ray photons emanate from the focal spot of the tube in all directions. They are then collimated to a rectangular field before passing through the patient and being recorded onto film or some form of digital image receptor. Projection images represent a shadow of the anatomical features that attenuate the beam between the tube and image receptor. A graphical representation of the two imaging modalities is shown in Figure 2.1.

![Diagram of projection-type and Computed tomography imaging](image)

*Figure 2.1: Diagram of projection-type and Computed tomography imaging as related to this experiment. Projection images are acquired using a stationary tube to direct a rectangular beam of radiation onto a photostimulable phosphor cassette. CT images involve processing of data acquired by a rotating X-ray tube and detector system.*
Computed tomography involves a rotating gantry that contains both an X-ray tube and digital image receptors that orbit about the patient. In this case, the divergent X-ray beam is collimated into a nearly 1-dimensional fan shape. The scan records these 1-dimensional images in rapid succession as the scanner completes each rotation (though multi-row scanners now effectively record 2-dimensional images). The temporal signature of 1-d image allows computers incorporated into the imaging system to reconstruct a tomographic slice based on the angle of the tube and positions of the pixels at each moment of exposure. This is completed on a ray-traced, line-by-line basis (backprojected) and repeated over multiple tomographic slices to reconstruct the patient’s 3-dimensional anatomy (4). Further computation may be involved to filter out the appearance of star-like artefacts due to the presence of highly-attenuating structures (5). In this case, the image –or series of images– is comprised of thousands of volumetric unit voxels; each voxel quantified by a density measurement given in Hounsfield Units (HU).

Figure 2.2: Diagram of image read-out process in Computed Radiography. A latent image is stored in the PSP cassette following exposure. During readout, phosphor crystals in each pixel’s area emit light in proportion to the amount of radiation absorbed. Light is converted into an electrical signal in the photomultiplier tube and subsequently into digital information.
2. Projection and Computed Tomographic Imaging

Modern radiographs are often captured digitally. Digital formats are common regardless of modality and these electronic images offer a number of advantages over traditional film images. The projection images captured in this study were recorded using a modality known as computed radiography. Computed radiography (CR) is a mode of imaging using filmless cassettes. In place of traditional photo-sensitive silver halide X-ray film, the cassettes contain a rigid plate with a thin coating of specialised photo-stimulable phosphor (PSP) material. When a radiographic exposure takes place, the incident X-ray photons are attenuated in the phosphor layer where they deposit energy (see Figure 2.2). In these specialised materials, some of the energy is lost as a lingering excitation event. Ionisations produced within the phosphor layer send an electron to a doping atom (Europium, in the case of the BaFBrI:Eu cassettes used for this experiment (109)), causing an electron to shift to a higher energy orbital. Importantly, the doped atom maintains this stable yet energetically unfavourable state until a secondary excitation occurs by laser light within the CR scanner. In this fashion, the plate stores information where the quantity of excited doping atoms in an area relates to the intensity of x-rays that deposit energy in that region (6). The scanner’s readout laser is tuned to a wavelength that induces a relaxation in the doping atoms, most often accomplished with a 633 nm He–Ne laser (110). When de-excitation occurs, the phosphor crystals emit secondary light photons of a different colour band which are selectively recorded by a photomultiplier tube (PMT). For the phosphor material Bariumfluorobromide (BaFBr), the maxima of the de-excitation spectrum occurs at 390 nm; easily distinguishable from the wavelength of excitation photons (110). The signal is digitized and converted to a grey level corresponding to the radiation dose for that pixel.

There are some important principles to keep in mind regarding this process of recording an image. The scanner detects secondary light photons emitted by the phosphor crystals, but if this phosphor layer is sufficiently thick light may spread out or be absorbed
within the material before being detected by the PMT (111). There is a tangible trade-off between image quality and detection-efficiency. For that reason, it is important to keep this layer of the imaging plate sufficiently thin to give good image resolution (usually 100-300 µm thick). The disadvantage of using a thin phosphor layer, on the other hand, is that it means many X-rays will pass through the photosensitive material undetected; particularly as one increases the tube potential and thus the penetrability of X-rays. This is worth consideration because surpassing the K-edge of gold, at 80.7 keV, requires the use of relatively high-energy X-ray photons.

![Figure 2.3](image.png)

Figure 2.3: Schematic of a Back-illuminated Photodiode (BIP) scintillator. Scintillating crystal (top layer) emits light photons which are converted into electrical signal by optically-coupled silicon p-i-n photodiode arrays. Recreated from (112)

Computed tomographic scanners, differing in how they geometrically acquire images, also record X-rays with a different type of detection system. Rather than recording X-ray energy as a latent image in specialised phosphorous material, CT scanners use scintillating materials that record X-ray photons and process the image in real-time. Like CR systems, scintillators convert X-ray photons into visible light and subsequently a digital
signal, but by considerably different means. The diagram in Figure 2.3 shows a schematic for a modern scintillator. In this case, the photons transmitted through the patient are absorbed by a layer of crystalline or ceramic scintillating material (such as caesium iodide, gadolinium oxysulphide, or gadolinium gallium garnet (Gd$_3$Ga$_5$O$_{12}$:Cr,Ce) (113).

The scintillation process first requires attenuation of transmitted radiation. Photoelectric absorption of an X-ray produces a mobile high-energy electron in one of the luminescent centres of the scintillating material. Relaxation occurs through ionisation and production of electron-hole pairs. Refilling of those electron holes by doped activator ions is associated with the release of a light photon by radiative decay (113). In this fashion, the scintillating crystals rapidly fluoresce, emitting light which is then captured by a photo-diode and converted to a small voltage potential. The greater the number of light photons emitted in a pixel’s area, the larger the voltage generated, which is converted into a correspondingly greater digital signal.

Because the CT gantry/tube system rotates very quickly (up to several times each second), these types of detectors must be able to capture images almost instantaneously. Any lag between the deposition of X-ray energy and detection of digital signal will result in an image that is not representative of the actual position of the X-ray tube and detector in relation to the patient (114). For that reason, the scintillating material must be chosen such that it captures X-ray energy and emits light within a few milliseconds or else the lingering “afterglow” will result in the appearance of artefacts when the image is reconstructed.

Efficient conversion of X-ray energy into light is important for reducing image noise and patient dose. Different scintillation materials vary in the amount of light output (114). The greater the number of light photons produced per unit of energy deposited by X-rays increases the signal produced by the photodiodes. Since scintillation relies on the production
and uninhibited transmission of light photons, the material should also be relatively translucent, allowing light photons to travel unattenuated to the photodiode.

Like the PSP plates in computed radiography, the X-ray absorbing layer in scintillating detectors must have high detection efficiency for diagnostic X-ray photons. In general, CT examinations are performed at higher tube potentials to provide enough X-ray penetration when the tube is angled to pass laterally through both the upper arms and torso. Computed tomography of the abdomen and torso is commonly performed at tube potentials between 120 and 140 kVp (2, 115). The detectors are designed to operate in this energy range and require greater cross-sectional thicknesses to sufficiently absorb the transmitted X-ray beam, even for highly penetrating photons up to 140 keV.

One of the aims of this study is to identify the appropriate energy range to maximise the detection of gold nanoparticles in radiology. Images are compared to samples of conventional iodinated CM to provide a reference against a material that is already clinically viable. Adjustment of energy range in this experiment is completed through manipulation of X-ray tube potential (though the effects of adding beam filtering material will be explored in some detail in Chapter 7. Figure 2.4 shows typical energy spectra for a clinical tungsten-anode X-ray tube (116). The K-edge of Au has been labelled. At 100 kVp, only a fraction of the X-ray energy is represented by photons above 80.7 keV. When the tube potential is increased further –up to 150 kVp– the relative percentage of X-rays exceeding the element’s K-edge is much greater. It is anticipated that optimal contrast enhancement relative to other structures (and iodine) will occur when utilising these high-energy X-rays.
Figure 2.4: X-ray energy spectra corresponding to several peak tube potentials between 50
and 150 kVp for a standard tungsten anode X-ray tube with 1 mm Al and 1mm Be inherent
filtration. The peaks near 58 and 67 keV correspond to tungsten characteristic K\textsubscript{\alpha} and K\textsubscript{\beta} X-
rays. The K-edge of gold has been labelled (dotted line) for reference.

2.2 Materials and Methods

Projection-type radiography and computed tomography involve the differential
attenuation of incident X-rays by materials between the focal point and image receptor. Such
attenuation causes variation in exit-beam intensity depending on position. The corresponding
shadows recorded on the image receptor are necessary to make a clinical diagnosis.

In this protocol, the average energy range and overall intensity of the x-ray beam may
be adjusted to optimise image quality by altering the peak tube potential (kVp) and electrical
current (mA), respectively. In the case of these measurements, the primary variable of
2. Projection and Computed Tomographic Imaging

Investigation is tube potential since, particularly with heavy elements, this has a pronounced effect on the attenuation of a given region. A suspension containing gold-nanoparticles was compared with a similar volume conventional iodinated contrast media in a series of phantom images. Radiographs were recorded using both projection and CT modalities to provide evidence representing the appropriate X-ray procedures to consider implementation of AuNP contrast media.

2.2.1 Contrast Phantom

A specialised x-ray phantom was constructed to hold the materials for this experiment. The phantom consisted of a rectangular acrylic [poly(methyl methacrylate), PMMA] plate, with dimensions of 129 x 10 x 45 mm as shown in Figure 2.5. PMMA is a well-suited material for use as a phantom since its composition and attenuation properties closely resemble those of human soft-tissue (117). Using a 4mm drill, 2 cm deep wells were created in one side. These wells were used to hold the CM samples during imaging.

Figure 2.5: Acrylic contrast phantom used in radiographic images. Experimental CM are loaded into 4mm diameter wells to simulate an intravenous volume.
Additional scattering material was added in both projection and CT imaging procedures. In order to simulate the normal attenuation and beam hardening by soft-tissue when taking a chest radiograph, the phantom was placed between 8 cm of PMMA scatter material (4 cm top and 4 cm bottom, see Figure 2.6). The use of a rectangular contrast phantom allowed the capture of these images with a minimal region of air gap to provide appropriate beam hardening and X-ray scattering. In CT imaging, a water phantom was employed to appropriately simulate a volume of abdominal tissue. This phantom consisted of a thin PMMA box filled with a volume of water to approximate soft tissue (Figure 2.7). The water phantom had dimensions of 100 mm (height) x 150 mm (length) x 150 mm (width).
2.2.2 Contrast media

Spherical AuNPs were obtained from Nanoprobe, Inc. (Yaphank, NY 11980, USA). Particles comprised of a black monoamine Nanogold® compound with an average diameter of 1.9 nm. 55 mg gold nanoparticle powder was suspended in 550 µL deionised water for imaging. Ultravist® (iopromide, Mallinckrodt Medical Pty, Ltd., Notthinghill, VIC 3168, Australia), a clinically-approved commercial contrast agent, was used for comparison. 1.075 mL stock (300 mg I per mL) was diluted with 3.925 mL deionised water.

For both image studies, projection and CT imaging, the contrast phantom was loaded with CM samples at equimolar concentration based on quantity of radiopaque atoms. In order to facilitate comparison between samples, a high concentration was chosen to maximise
contrast signal relative to image noise. A concentration of 0.5077 M (moles per litre) was utilised. This corresponds to density concentrations of 100 mg Au per mL (9.1% w/v) [gold] and 64.5 mg I per mL (6.1% w/v) [iodine]. It is important to note that equimolar comparison of gold and iodine was utilised only for this image study. The results from subsequent radiographic and simulation studies (Chapters 5-8) compare Au and I at equal densities.

To illustrate the effect of image receptor type, a follow up study was conducted with CM samples at equal concentration by mass: 120 mg radiopaque element per mL (10.7% w/v). Those results are given in section 2.3.3 “Effect of the Image Receptor”.

### 2.2.3 Adjustment of Tube Potential

The aim of investigating contrast enhancement dependent on energy range relied on taking a series of images at varying peak tube potentials. Projection images were collected using a Shimadzu R-20 X-ray tube (Shimadzu Inc., Kyoto, Japan.), recorded on Kodak Industrex® GP phosphor cassettes and processed on a Kodak DirectView CR900 scanner (Eastman Kodak Inc., Rochester, NY, USA). In CR images, tube voltages ranging from 40 to 80 kVp in 10 kilovolt increments have been reported below. This range was limited by the equipment’s minimum voltage. Although the tube could be utilised at potentials as high as 130 kVp, it was observed that the image receptors showed poor photon absorption above approximately 80 keV. Results from an exposure at 120 kVp, however, are reported below to illustrate this phenomenon. CT images were captured at potentials between 80 and 140 kVp in 20 kilovolt increments using a GE LightSpeed RT single-slice CT scanner (GE Healthcare, Waukesha, WI, USA).

The energy range tested corresponds with several common clinical procedures. Projection images represent a range of potentials utilised in chest radiography and
angiographic imaging. Typical chest x-rays are captured at peak kilovoltage values of roughly 100 kVp, while angiography is usually done in the range of 70 to 90 kVp depending on equipment and patient size. Low energy images (around 40 kVp) can be used to draw some conclusions about contrast-enhancement in mammography, although most mammographic machines operate at even lower energies (around 30 kVp) and with specialised combinations of anode target material and filters that further optimise the energy spectrum to produce contrast in breast tissue (5). CT studies are often performed at potentials between 120 and 140 kVp.

2.2.4 Analysis of Contrast-to-Noise Ratio

Evaluation by contrast-to-noise ratios (background Perspex to contrast medium) is the standard descriptor for contrast resolution in digital imaging (4) and was chosen to standardise the methodology of comparison between modalities. Contrast values \((I_{cm} - I_{bg}, \Delta I)\) were divided by the amplitude of image noise in a set of background ROIs \((\sigma_{bg})\) to calculate CNR:

\[
CNR = \frac{I_{cm} - I_{bg}}{\sigma_{bg}}
\]

(2-1)

Where \(I_{cm}\) is the image intensity in the region overlaying the contrast medium, \(I_{bg}\) is the mean intensity of the adjacent background regions, and \(\sigma_{bg}\) is the standard deviation in intensity in the background ROIs.

In projection imaging, one image was taken at each potential. 0.8 mm square ROIs were chosen (49 pixels). Contrast medium ROIs correspond to the centre of the CM sample (greatest thickness, ~ 4 mm). Two adjacent background ROIs were chosen for subtraction. Mean gray value for the CM and background regions was subtracted and divided by mean image noise to yield CNR values. Each image produced 18 separate area sets for sampling (n=18).
2. Projection and Computed Tomographic Imaging

CT images were captured as a series of axial slices with a total of 17 images available for comparison (n=17). Circular ROIs (corresponding to the cross-sectional shape of CM wells) were chosen comprised of 52 pixels (2.8 mm diameter in images). For each contrast medium sample, one ROI was selected overlaying the centre of the CM \( I_{cm} \) area and 8 background ROIs \( I_{bg}, \sigma_{bg} \) were chosen in the adjacent Perspex. Image intensity was determined from voxel HU values and CNR was calculated according to the equation (2-1).

2.2.5 Evaluation of Nanoparticle Aggregates by Electron Microscopy

After a one month period inside the PMMA contrast phantom, the gold nanoparticle suspension was removed. 50 \( \mu \)L of suspension was dropped onto carbon-coated copper grids and left overnight to evaporate. TEM (Transmission Electron Microscope) micrographs were collected on a JEOL JEM 1010 Microscope (JEOL Ltd., Tokyo, Japan) at 100 keV. Images were recorded onto film and subsequently digitised for analysis.

2.3 Results and Discussion

2.3.1 Analysis of contrast-to-noise ratio

Image studies (Figure 2.8) show that the variation in contrast enhancement between AuNPs and iodinated CM is dependent on the potential used. Figure 2.8 shows images with both exploratory substances, gold nanoparticles and iopromide, inside the image phantom. Projection CR images show contrast samples from a lateral view while CT images display cross-sectional slices of the CM-bearing wells. Contrast-to-noise ratios for contrast media in projection imaging are presented in Table 2.1 while data from CT images at higher tube potential values are shown in Table 2.2. CNR values are evaluated for difference in mean by two-sample, one-tail t-test at each potential (assuming equal variance). In each image set,
gold at equimolar concentration displayed significantly greater CNR than iodinated CM (P<0.05). Details of statistical analyses are given in appendix 10.5.1.

![Projection and Computed Tomographic Imaging](image)

**Figure 2.8**: Radiographic images of gold nanoparticle CM and Ultravist (iopromide) iodinated CM. CR (projection) images are recorded for potentials between 40 and 80 kVp. Tomographic images (CT) are shown for potentials between 80 and 140 kVp.

<table>
<thead>
<tr>
<th>Tube Potential (kVp)</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>100</th>
<th>120</th>
<th>140</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ΔI AuNP</td>
<td>97.68</td>
<td>62.48</td>
<td>46.28</td>
<td>38.21</td>
<td>32.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ΔI Iopromide</td>
<td>50.57</td>
<td>45.76</td>
<td>37.33</td>
<td>31.57</td>
<td>28.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CNR AuNP</td>
<td>9.73</td>
<td>10.52</td>
<td>9.50</td>
<td>8.15</td>
<td>6.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Deviation AuNP CNR</td>
<td>0.64</td>
<td>0.62</td>
<td>0.85</td>
<td>0.59</td>
<td>0.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CNR Iopromide</td>
<td>5.17</td>
<td>7.89</td>
<td>7.77</td>
<td>6.84</td>
<td>5.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Deviation I CNR</td>
<td>0.28</td>
<td>0.44</td>
<td>0.46</td>
<td>0.54</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage difference in mean CNR (Au vs I)</td>
<td>( \frac{CNR_{Au} - CNR_{I}}{CNR_{I}} \times 100% )</td>
<td>88.00</td>
<td>33.27</td>
<td>22.356</td>
<td>19.02</td>
<td>13.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.1**: Contrast-to-Noise ratio analysis for experimental contrast media (gold and iodine) in CR imaging. Tube potentials vary between 40 and 80 kVp.
2. Projection and Computed Tomographic Imaging

Figure 2.9: Measured Contrast-to-noise ratios for AuNP and iodinated CM samples in projection (CR) images. CNR values for tube potentials between 40 and 80 kVp are shown. Note how contrast of iodinated CM increases relative to Au as tube potential increases over the K-edge of iodine (33.2 keV). Error bars represent standard deviation*. Each data point corresponds to the mean CNR of n=18 measurements per CM sample. There is a statistically significant difference in CNR values at all tube potentials ($p<0.05$).

It is evident that contrast enhancement in regions containing AuNP suspension is greater than that for iopromide at low tube potentials. At 40 kVp, the CNR value for AuNPs is 88±17% greater than that for conventional CM. With increasing tube potential, the difference between samples is shown to decrease. At 50 and 60 kVp, AuNPs display 33±8% and 22±12% superiority, respectively. This trend continues up to 80 kVp, where there is only a 14% difference in SNR between materials. Relative CNR values at 80 kVp are consistent in both CR and CT imaging, showing only 13.2 and 6.8 % higher contrast for gold samples than iodine. It is worth noting that the amplitude of the CNR values for a given image is highly dependent on the selected mAs and its related dosage at the image receptor. For this reason,

* note: all error bars in this thesis represent standard deviation
the general shape of Figure 2.9, with a distinct peak for either CM in the range of 45-55 kVp, may not be representative of the optimal tube voltage for detecting the particular contrast agent in a clinical setting.

Figure 2.10: Measured Contrast-to-noise ratios for AuNP and iodinated CM samples in computed tomography (CT). CNR values for tube potentials between 80 and 140 kVp are shown. Relative enhancement of AuNPs markedly increases with increasing potential; attributed the X-ray tube emitting photons above the Au K-edge (80.7 keV). Again, equimolar AuNP CM samples display statistically greater CNR values than iodinated CM at all tube potentials ($p<0.05$, $n=17$ per datapoint)

As tube potential is further increased above 80 kVp, AuNPs again display greater attenuation than iodinated CM (shown in findings from CT images). At 100 kVp, CNR for AuNPs is 49±16% greater than iopromide. At 120 kVp improvement increases to 78±30% and greatest superiority is found at 140 kVp where the contrast-to-noise ratio for gold nanoparticles is 113±25% higher. At the concentrations used in this image study –equal by moles of radiopaque atom– gold shows a statistically significant improvement in CNR at all potentials ($p<0.05$). Contrast values are highly consistent within each image set for a selected
2. Projection and Computed Tomographic Imaging

kV value. Fluctuation in noise between measurements is the predominant source of variation in CNR values.

<table>
<thead>
<tr>
<th>Tube Potential (kVp)</th>
<th>80</th>
<th>100</th>
<th>120</th>
<th>140</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ΔI AuNP (HU)</td>
<td>2677.40</td>
<td>2714.11</td>
<td>2705.94</td>
<td>2594.81</td>
</tr>
<tr>
<td>Mean ΔI Iopromide (HU)</td>
<td>2453.34</td>
<td>1862.18</td>
<td>1475.74</td>
<td>1240.15</td>
</tr>
<tr>
<td>Mean CNR AuNP</td>
<td>96.86</td>
<td>149.56</td>
<td>182.26</td>
<td>211.27</td>
</tr>
<tr>
<td>Standard Deviation AuNP CNR</td>
<td>8.35</td>
<td>11.53</td>
<td>21.85</td>
<td>20.26</td>
</tr>
<tr>
<td>Mean CNR Iopromide</td>
<td>90.69</td>
<td>100.70</td>
<td>102.37</td>
<td>99.32</td>
</tr>
<tr>
<td>Standard Deviation I CNR</td>
<td>9.69</td>
<td>9.87</td>
<td>6.65</td>
<td>10.72</td>
</tr>
<tr>
<td>Percentage difference in mean $CNR$ (Au vs I)</td>
<td>$6.80$</td>
<td>$48.52$</td>
<td>$78.04$</td>
<td>$112.71$</td>
</tr>
</tbody>
</table>

Table 2.2: Analysis of Contrast-to-Noise ratios for gold and iodine based contrast media in CT imaging. Potentials selected between 80 and 140 kVp

2.3.2 Energy Range

Compared to the conventional contrast media, we have shown that gold nanoparticles display much greater attenuation for applications employing low tube potentials (<50 kVp) such as mammography or biological imaging of small animals. In this range, attenuation occurs primarily by photo-electric effect. The probability of this interaction is highly dependent on a material’s atomic number ($\Delta I \propto Z^3$). In these energy ranges, gold (Z=79) is a more suitable radiopaque element than iodine (Z=53). It is also speculated that the probability of absorption by photoelectric effect is well suited towards the L-edge of gold, 11.9 keV, when relatively low peak kilovoltages are selected (10).
At potentials studied in the range of coronary angiography (70-90 kVp), we have identified that both AuNPs and iopromide will produce similar visualisation. In this range, iodine’s K-edge occurs at the optimal energy, 33.2 keV, to absorb image-resolving photons. That means that a large proportion of X-rays emitted by the tube have wavelengths that correspond with energies slightly exceeding that 33.2 keV threshold. In this case, the iodine atoms will be absorbing more incident X-rays by photoelectric effect, while gold – compensated by a greater electron density– will rely more heavily on attenuation by Compton scattering.

If the X-ray tube potential is increased further (above approximately 100 kVp), the impact of the Au K-edge is apparent. In tomographic imaging, increasing tube potential led to a decrease in contrast for iopromide. This is attributed to a larger fraction of X-ray photons occurring above 100 keV in energy where the attenuation coefficient of iodine has declined to roughly one twentieth of the value at the element’s 33.2 keV K-edge. Gold nanoparticle samples, on the other hand, exhibited a nearly constant HU density in CT imaging regardless of potential. As a rule, the HU value for high density materials decreases with increasing kVp (118). Contrast attributed to the presence heavy of elements like gold must be predicted differently because of the material’s comparatively high K-edge. These results show that the increase in absorption above 80.7 keV maintains strong attenuation properties at potentials as high as 140 kVp.

This data falls roughly in line with known mass attenuation coefficients for bulk elemental media. We cannot confirm findings by Kim et al. of 5 times greater attenuation using AuNPs compared to iodine 120 kVp, however a two to three-fold increase in visualisation is attainable (66). That is supported by tabulated mass attenuation data showing that gold has 2.6-2.7 times greater mass attenuation coefficient values for photons above 80.7
2. Projection and Computed Tomographic Imaging

keV (9). It is anticipated that gold would continue to efficiently attenuate incident X-rays at peak kilovoltage settings above 140 kVp.

The use of high energy X-rays in imaging can reduce patient absorbed dose while maintaining the same optical density at the image receptor. Sandborg et al. discuss the effect of adjusting tube potential on image quality and patient radiation exposure (119). Their findings indicate that the sacrifices in terms of reduced image contrast and increased scatter become too significant above a certain tube potential to justify the reduction in patient dose. This is described by calculating the mean absorbed dose at a variety of potentials to achieve a constant CNR value. Those results, however, are based on the detection of features such as bone and air. Attenuation of high-energy photons by gold, on the other hand, would increase the optimal tube potential to achieve a high quality image with minimal patient dose.

2.3.3 Effect of the Image Receptor on detection of radiographic contrast from gold nanoparticles

The process of radiographic image formation can be divided into three discrete stages: photon generation, differential attenuation, and detection. An ideal image involves the optimisation of all three components to produce a high-quality image with minimal patient radiation dose. The peak tube potential (kVp) and filtration should be chosen to provide good subject contrast (differential attenuation) without unnecessary radiation exposure. Equally important, however, is the need to detect the transmitted X-rays in all regions of the image. Each transmitted photons represents a sampling that describes the patient’s tissue density and elemental composition for a given area (based on whether it is attenuated or transmitted). Maximising the detection of these photons will likewise maximise the acquisition of information for a given exposure. These highly-penetrating photons are of particular value when it is considered that they are represented by only a small portion of scattered X-rays.
2. Projection and Computed Tomographic Imaging

that contain no information. Compton scattering produces secondary photons with reduced
energy and penetrating power. Accordingly, scatter radiation is unlikely to transmit through
an X-ray detector without being recorded.

![Images of exploratory Contrast Media at 120 kVp](image)

**Figure 2.11**: Images of AuNP and Iodinated CM at 120 kVp collected using computed
radiography (bottom) and computed tomography (top). Iopromide displays superior contrast
enhancement in CR imaging, while AuNPs are more clearly visualised in CT.

![Figure 2.11](image)

**Figure 2.12a & b**: Contrast-to-Noise ratios for AuNP and iodinated CM compared across two
modalities (CT and CR) and 120 kVp. In computed tomography, the relative appearance of
gold is 57% greater than iodine, while in computed radiography it is 12% lower in spite of
similar imaging conditions. There is a significant difference between mean CNR values in
both instances ($p<0.05$, for each sample $n_{CR}=12$, $n_{CT}=11$).
In the process of analysing the data from the projection (CR) images, surprisingly low contrast was shown at high tube potentials when comparing enhancement of AuNP CM relative to iodinated contrast media. Even above 100 kV (well surpassing the K-edge of gold), AuNP CM at equivalent density did not display any improvement compared to iodine. When a similar image set was taken using a CT scanner, however, greater contrast enhancement was visualised for AuNPs compared to iodinated CM. Here we discovered an interesting phenomenon. Figure 2.11 shows the same AuNP and iopromide samples in images recorded at 120 kVp, but with the different imaging techniques. When the same samples were radiographed at the same energy with different modalities, gold provided more contrast in CT while iodine provided a greater mean CNR value in CR. The corresponding CNR values are shown in Figure 2.12a & b. Note that in projection imaging, the iodinated CM has a greater mean CNR value than shown by the gold nanoparticle sample (t-test, $p=0.0040$). In CT imaging, the opposite trend is shown with gold displaying significantly greater contrast ($p=0.0000003$).

To some degree, this can be attributed to increased beam hardening in the larger CT water phantom, and perhaps a slightly different voltage waveform in the CT X-ray tube compared to the Shimadzu projection unit (4). Examination of the absorption coefficient and coating weights of the particular image receptors used in either CT or CR indicates that X-ray detection may play a large role in the discrepancy between CNR values. The same contrast samples were used in both imaging procedures and captured only a matter of days apart (computed radiography study: 1st of June, 2007, CT study: 6th of July, 2007). We cannot attribute the decreased enhancement to aggregation of gold nanoparticles in the CR images because this study was completed prior to the computed tomography experiment. Nor was there any evidence in either image set of inhomogeneous, high-density regions that would be indicative of nanoparticle aggregation. The greatest difference between these imaging
modalities that could be attributed to a bias for one high-Z CM material was the type of image receptor.

In this case, we are not concerned with the sequential difference between how the image receptors recording information. In both cases, computed radiography and computed tomography, the challenge is to convert transmitted X-ray energy into a digital signal. The fact that one relies on rapid scintillation and the other stores a latent image for subsequent readout is irrelevant. Instead, the importance lies in the ability of the different image receptors to photoelectrically absorb X-ray photons, particularly those of high-energy which surpass the K-edge of Au.
2. Projection and Computed Tomographic Imaging

In this case, the images were recorded on a Computed Radiography system with photostimulable phosphor (PSP) cassettes. These types of cassettes contain Barium Fluoro Bromine (85%) with Iodine (15%) doped with Europium. Due to the nature of readout in a CR system, the phosphor layer thickness must be minimised to reduce light spread and maintain good resolution. The highest potentials used in CR examinations are for Chest X-rays in the range of 100 kVp. The PSP material is chosen to attenuate a large percentage of incident photons at these potentials, but absorption drops substantially above those values and
it is particularly susceptible to fogging by scatter radiation. In the Kodak Industrex® plates used in this examination, only about 25% of incident photons at 80 keV will interact with the image-forming layer. That percentage continues to decline at higher energies (120).

Scintillators have markedly better detection efficiency than phosphor plates. This is described by the image receptor’s detective quantum efficiency (DQE), which incorporates the imaging system’s ability to efficiently translate subject contrast into a high resolution, noise-free image. The scintillating detectors used in CT imaging have distinctly higher DQE values than other radiographic image-capture modalities (5). Flat-panel digital detectors have a DQE as high as 65% (121), while the values for PSP plates and screen-film systems are approximately 35% (122) and 25% (123), respectively.

Let us consider that both imaging modalities result in equivalent primary images. That is, in each case the same energy spectrum of incident X-ray photons (120 kVp) has been attenuated by regions of soft tissue and CM to produce the same pattern of transmitted radiation intensity prior to registration within the image receptor. Figure 2.13 shows the fractional absorption versus X-ray energy for photons incident on either PSP cassettes or a Caesium iodide scintillator [PSP phosphor material BaFBrI:Eu, $\rho = 3.21$ g/cm$^3$ (124), thickness $= 300$ µm (109) Scintillator material CsI, $\rho = 3.38$ g/cm$^3$ (with 75% assumed packing density) (125), thickness $= 2.0$ mm (126)]. It is evident from this chart that the CsI scintillator absorbs a much greater fraction of X-ray photons for energies above 40 keV. At 100 keV, though absorption in the scintillator has declined to 72.8%, the value for the BaFBrI phosphor is much lower, at only 13.5%. That means that the scintillator is recording over five times more of the available photons in this energy range than the PSP cassette (the same range where gold has a 2.6-2.7 times greater attenuation coefficient). Most of the exposure to the CR cassettes, instead results from energy deposited by photons below roughly 60 keV in energy, regardless of whether a high tube potential is selected.
2. Projection and Computed Tomographic Imaging

We suggest that the image receptor should be considered prior to implementing gold nanoparticles in a radiographic procedure. Though contrast at potentials below 50 kVp (utilising the 11.9 keV L-edge of Au) can be efficiently detected with PSP cassettes, it is impractical to use this form of image receptor at higher energies to record the appearance of AuNPs. An image receptor with good fractional absorption for photons above 80.7 keV should be considered to maximise the appearance of gold nanoparticles procedures at tube potentials above 100 kVp.

2.3.4 Nanoparticle Aggregation & Stability

In order to be clinically viable, contrast agents should be introduced into the body in a concentrated form. After injection, CM is diluted by the roughly six litres of blood in adult humans. Even in this dilute state, after flowing through the bloodstream for several minutes, a contrast agent must contain a sufficient density of radiopaque element to attenuate the beam of incident x-rays by a measurable amount. There is a limitation in the volume of contrast medium solution that can be administered. The notion of injecting an extra litre of fluid into the circulatory system is impractical. Instead, CM molecules are designed to be highly soluble. That permits a large mass of radiopaque element to be concentrated into a relatively small volume of solvent; thereby decreasing the amount of liquid that must be administered.

Solubility is one of the major hurdles in designing a highly-concentrated colloid CM. During this experiment, the issue was seen first-hand. At the outset of the project, it was apparent that it would be difficult to synthesise hydrophilic nanoparticles that could be concentrated or dried and resuspended. This is particularly the case if one hopes to synthesise particles at a scale above a few milligrams of gold. Instead we utilised gold nanoparticles from an outside supplier (Nanoprobes Inc.) that had been specifically designed for high solubility in aqueous solvents. Our experience with this material indicated that the particles
were highly soluble in water, permitting concentrations of at least 20% w/v, but the resulting suspension was only stable for a matter of days. After that time period inhomogeneity in the suspension was apparent, and sandy spots (large enough to be visible to the naked eye) were observed.

Figure 2.14 shows a radiograph of CM samples one month after being loaded into a contrast phantom. Iopromide is shown on the left and its visualisation is highly uniform (less the presence of air bubbles near the top of the CM well). Alternatively, contrast in AuNP well as noticeably decreased over most of its area in the recorded image. Instead it is marked by small regions of high density, most notably in a spot near the base. Efforts to resuspend the nanoparticles by agitating with vortex mixer and ultrasonication were unsuccessful, indicating that these particles had irreversibly aggregated.
Figure 2.14: Image of conventional iodinated contrast media (left in radiograph) and a suspension of gold nanoparticles (right) that has irreversibly aggregated. The iodinated species remains highly homogeneous after several weeks while the gold has collected in distinct bands at the steps of this contrast phantom. Image of the phantom is inset to provide scale.
Figure 2.15: Large, aggregated cluster composed of several hundred-thousand 10nm gold nanoparticles

Transmission Electron Micrographs indicate the nature of the aggregating particles. Figure 2.15 shows a particularly large cluster as viewed at 15,000x magnification. Here individual particles can be discerned near the margins of the structure (magnified in inset). Based on the diameter of the individual particles relative to the volume of the cluster, it can be estimated that this aggregate contains upwards of one million individual AuNPs.
A second TEM image, Figure 2.16, shows the presence of individual particles and two different cluster types. In this instance, some clustering is occurring to form aggregates with a roughly spherical shape. There are also instances where nanoparticles are collecting in long strands. Shipway et al. describe the formation of these cluster types based on the presence of cations on the nanoparticle surface (127). Multiply charged aggregates –those with more than one positively charged group– tend to form dense, spherically-shaped clusters. Singly-charged aggregates form as strands with nanoparticles collecting end-to-end. These types of strands have also been reported to occur in PEG-stabilised gold nanoparticles by Eck et al. (128).
2. Projection and Computed Tomographic Imaging

It has been proposed that aggregation of metallic nanoparticles occurs through the formation of weak chemical bonds (127, 129). Concentrating these suspensions results in the confinement many particles into a very small space. Inevitably this causes the surfaces of neighbouring particles to interact. In the case of AuNPs, the primary force between particles is electrostatic repulsion because the surface groups share the same small negative charge. If the particles are limited to a small volume, however, there is insufficient space to continuously maintain the minimum distance of repulsion between surface groups. This may result in the displacement of an electron from one group to form a chemical bond (129).

2.4 Conclusions

This phantom study indicates that improved image quality can be achieved using AuNP contrast media compared to iopromide. At a concentration of 0.5077 M, AuNP contrast enhancement is up to 88% greater than iodine at low energies and up to 115% greater at high energies according to SNR. We have determined that there will be no significant improvement in contrast compared to iodine at moderate tube potentials (70-90 kVp). Alternatively, comparable enhancement levels should be possible in certain settings with lower molar concentrations of gold compared to iodine. These findings provide further evidence for practical applications of AuNPs in contrast-aided procedures. Results of measurement are in accordance with tabulated attenuation data from NIST (10). This study has also indicated importance of the image receptor on detection of high-energy X-rays that are differentially attenuated by regions containing AuNPs and those in the background. Computed tomography shows better results in that respect and we expect that this modality would make more efficient use of gold nanoparticle CM than projection CR imaging when selecting high peak kilovoltages.
3 X-ray Energy analysis

From the initial experiments to quantify contrast enhancement using AuNPs, it was evident that adjustments of tube potential had a significant impact on the visualisation of gold. An in-depth analysis of the attenuation of X-ray beam was warranted to fully investigate the observed changes in contrast enhancement, particularly because the K-edge of Au occurs at a high energy relative to range of X-rays employed in diagnostic imaging. The aim of such experimentation is to confirm that the appearance of gold relative to iodinated occurs according to tabulated attenuation coefficients (9, 10). This experiment was the first step towards a comprehensive mathematical analysis (and eventually computational modelling) of attenuation and contrast enhancement of gold atoms in a diagnostic radiology system.

3.1 Introduction

Adjusting tube potential results in a shift in average energy of the polychromatic photon beam emitted by an X-ray tube (shown previously in Figure 2.4). By increasing the kinetic energy of electrons prior to interacting with the tungsten atoms in the anode target, these accelerated electrons have a greater amount of energy to donate as Bremsstrahlung radiation. The X-rays produced have, on average, more energy as described by a shorter mean wavelength or higher frequency (6).

Moreover, electrons that exceed the binding energy of K- and L- shell orbitals in tungsten have the potential to expel the atom’s bound electrons and create a vacancy. This vacancy is rapidly refilled with electrons from a higher orbital: for example, an L₂-shell electron shifting to fill a vacant K-shell orbital. This process releases energy in the form of electromagnetic radiation (a characteristic X-ray). The frequency of the emitted photon is
determined by the difference in binding energies of the two electron orbitals involved:
\[ h\nu = E_K - E_{Li} \]
where \( h \) is Planck’s constant, \( \nu \) is the frequency of the characteristic X-ray, \( E_K \) is the binding energy of the K-shell orbital, and \( E_{Li} \) is the binding energy of the L\(_{II}\)-shell orbital (note: the K- and L- orbitals shown have been chosen to illustrate this example, refilling may actually occur from any higher orbital: L\(_{III}\), M, etc.) (4). Because the electron orbitals occur at discrete energies for a particular atom, X-rays produced in this fashion always have one of several discrete frequencies that are “characteristic” of that element. In the case of tungsten, K\(_{\alpha}\) characteristic X-rays have energies of either \( K_{\alpha 1} = 59.321 \) or \( K_{\alpha 2} = 57.984 \) keV, corresponding to the K-shell being refilled by either the L\(_{III}\) or L\(_{II}\) orbital, respectively. Tungsten characteristic peaks between 66.95 and 69.08 keV correspond to refilling from higher orbitals (M, N, etc.) (130). Increasing the quantity of electrons with sufficient kinetic energy to eject Tungsten’s K- and L-shell electrons also results in an increase in the quantity of resultant characteristic X-rays that are emitted.

It is often conventional to describe an X-ray beam based on its peak potential or half value thickness (HVT), but when quantifying attenuation by materials that have comparatively high K-edge values, such an approximation is insufficient. The increased probability of absorption by photoelectric effect above the K-edge of heavy elements (\( Z > ~30 \)) is one of the principle processes that leads to high radiopacity and visualisation in an X-ray image. The K-edge effect occurs contrary to the general trend of decreasing attenuation (greater beam penetration) when using high energy photons. Characterising an X-ray beam’s fluence based on its energy spectrum allows calculation of attenuation according to the Beer-Lambert Law using equation (1-9) derived in section 1.3 “Radiographic Image Contrast”:

\[
I = \left[ I_0(kVp, E) * e^{-\sum \frac{\mu(E, Z)\rho(E, Z)}{\rho(E, Z)}} \right] dE
\]

(3-1)
3. X-ray Energy analysis

The mass attenuation coefficient of a material is dependent on the energy of an incident photon. The attenuation coefficients have been meticulously quantified by the National Institute for Standards and Technology for the first 92 elements of the periodic table at energies between 1 keV and 20 MeV (10).

In order to fully evaluate the attenuation by the elements of interest, gold and iodine, we needed to first acquire accurate energy spectra to indicate the operational energy range of the radiographic equipment being used. For that purpose, we utilised a Cadmium Telluride X-ray/gamma ray detector. Much like a traditional ionisation chamber, this instrument can record the intensity of an incident X-ray beam by measuring the energy deposited in its surface. Unlike an ionisation chamber, the CdTe material in this type of detector acts as a large band-gap semiconductor which records energy deposition very rapidly. This conversion occurs so rapidly, it can be scored on a photon-by-photon basis even with the high levels of photon fluence produced by a diagnostic X-ray tube. Each photon is registered as a small voltage “pulse”, in which the amplitude is proportional to the energy of the incident photon (though scatter correction, electron-stripping and pulse-height discrimination algorithms are necessary to maintain accurate measurements (131) (132)). When the detector is combined with a multi-channel analyser, this system can produce a histogram of the number of X-rays divided over large number of different energy range “bins”. The energy level of the channels is calibrated to known gamma ray peaks produced by radionuclide sources. The device can then create histograms that are highly representative of the polychromatic X-ray spectrum emitted during a radiographic exposure.
3. X-ray Energy analysis

3.2 Materials and Methods

3.2.1 CdTe Detector

X-ray energy spectra were recorded with a CdTe detector coupled to a Multi-Channel analyser (XR100CDTE and PX4, Amptek, Inc., Bedford, MA 01730, USA). In order to accommodate the high rate of X-ray output from the X-ray tube, fast detection settings were used (rise time: 3.2 μs, top time 0.4 μs, fast threshold: 150, pile-up rejection: enabled). X-rays below 15 keV have been ignored to improve spectrum resolution. Due to their low penetrating power, the effect of photons with such low energy can be considered negligible in terms of image quality. Spectra were collected over 1024 energy bin channels. Each channel corresponded to 0.959 keV energy increments. Calibration of detector channels to energy levels was completed with $^{241}\text{Am}$ (59.5 keV), $^{137}\text{Cs}$ (661 keV), and $^{22}\text{Na}$ (511 keV) gamma ray sources.
3. X-ray Energy analysis

Figure 3.1: Cadmium Telluride X-ray detector being aligned for spectral acquisition of the Shimadzu R-20 diagnostic X-ray tube. Inset: Close-up of CdTe detector with 8.4 cm PMMA beam-hardening material and 10 µm pinhole camera
3. X-ray Energy analysis

X-ray energy spectra were captured from a Shimadzu R-20 X-ray tube (Shimadzu Inc., Kyoto, Japan). To reduce X-ray fluence at the detector surface, energy spectra from the divergent X-ray beam were collected at the maximum focus film distance permissible: 186 cm (note: acquisition of beam at a horizontal angle was not successful due to issues aligning detector and collimator with the beam’s central ray). Tube current was set to the minimum, small-focal spot value of 50 mA. Further reduction of X-ray intensity was achieved through collimation. Beam area was reduced by inserting a 10 µm pinhole camera in line with the X-ray beam. The X-ray tube was aligned with a spirit level to achieve a perfectly vertical central ray. The detector-collimator apparatus was then oriented with the central ray and levelled accordingly using thin aluminium shims (see Figure 3.1).

Energy spectra were captured after passing through 8.4 cm PMMA scatter material (7.12 mm thick sheets). The addition of beam-hardening material served two purposes. It filtered out a large quantity of incident photons, improving resolution of the captured X-ray energy spectra. Discussion of issues with energy resolution and limitations of the CdTe detector are given in section 3.3.2. Moreover, photons passing through this thickness of tissue-simulating material are representative of the energy range of X-rays available for detection in a radiograph collected at the selected tube potentials. Low energy X-rays are predominately attenuated in the patient prior to possible detection in the image receptor. The thickness, 8.4 cm, is equivalent to the amount of scatter material used in the projection images discussed in chapter 2. Each reported energy spectrum is compiled from 5 separate 160 mAs exposures to reduce the appearance of noise in the resolved spectrum.

3.2.2 Calculation of attenuation by contrast media

The attenuation of measured X-ray spectra was calculated using known mass attenuation coefficients and the Beer-Lambert exponential attenuation equation (3-1). The
3. X-ray Energy analysis

addition of CM atoms to the phantom structure is considered as having no impact on the thickness or composition of background material. In this way, transmission of X-rays through a CM region is altered only by the increased attenuation by a certain thickness and density of radiopaque atoms. Given that the energy spectra recorded with CdTe detector are equivalent to the transmission through a background region, $I_{bg}$, the transmission through a region of contrast media, $I_{cm}$, can be calculated using tabulated mass attenuation data. Thus:

$$I_{cm} = \int_{0}^{kVp} I_{oE}(kVp, E) \cdot e^{-(\mu / \rho)_{cm}(E, Z) \rho_{cm}X_{cm}} dE$$

(3-2)

$$I_{bg} = \int_{0}^{kVp} I_{oE}(kVp, E) dE$$

(3-3)

Where: At a selected tube potential $kVp$, $I_{oE}$ is the incident X-ray intensity for a given photon energy, $E$, which is taken from the measured CdTe spectra. $(\mu / \rho)_{cm}(E, Z)$ is the mass attenuation coefficient for the particular CM element, Z, (either Au or I). $\rho_{cm}$ is the density of radiopaque material in the contrast media ($\rho_{Au} = 0.1 \text{ g/cm}^3$, $\rho_{I} = 0.065 \text{ g/cm}^3$ from section 2.2.2). $X_{cm}$ is the thickness of CM sample (0.4 cm for both materials).

The intensity of the X-ray beam in a background ROI is taken as the intensity of the integrated CdTe spectrum as shown in equation (3-3). Image contrast can be estimated based on the variation in transmitted X-ray intensity of these regions. This calculated image contrast can be approximately compared to the measured radiographic contrast values from previous image studies (Chapter 2 Projection and Computed Tomographic Imaging). Though contrast-to-noise ratio cannot be estimated from such a technique, the relative difference in attenuation between gold and iodine can be compared to the measured difference in contrast enhancement. Given that image contrast can be calculated:

$$\Delta I = I_{cm} - I_{bg}$$

(3-4)
3. X-ray Energy analysis

It is possible to compare the calculated percentage variation in attenuation between CM materials:

\[
\frac{\Delta I_{Au} - \Delta I}{\Delta I} \times 100\% \quad (3-5)
\]

to the measured percentage variation in contrast enhancement for CM samples from previous image studies:

\[
\frac{CNR_{Au} - CNR}{CNR} \times 100\% . \quad (3-6)
\]

3.3 Results and Discussion

3.3.1 Estimation of X-ray Absorption by Contrast Media

X-ray photon distributions for beams at various potentials are shown in Figure 3.2. It is evident that the presence of scatter material removes a large proportion of low-energy photons (for comparison tabulated to spectra with only inherent filtration see Figure 2.4 or Figure 6.3). The expected energy of maximum intensity for an unattenuated spectrum (assuming only inherent filtration) occurs between one third and one half of the selected tube potential (6). The presence of 8.4 cm of tissue-simulating material raises the modal energy. At 70 kVp, peak intensity is found at approximately 65 keV, while at 120 kVp it occurs at 90 keV. In general, results show that scatter material selectively removes photons below 60 keV in energy. This indicates that the majority of x-ray photons contributing to the recorded image occur above this energy value.
Data collected from images are supported by theoretical attenuation of measured x-ray energy spectra. Figure 3.3 & Figure 3.4 show the attenuation of measured spectra at 80 and 110 kVp by the exploratory radiopaque elements. Attenuation is tabulated according to known elemental attenuation coefficients at the concentrations and dimensions used in the above image study (10). The spectrum of attenuated photons, shown in grey, represents the difference in measured and theoretically estimated transmission spectra. At 80 kVp (Figure 3.3) both samples have similar overall attenuation. This is in agreement with findings from prior image studies using projection and tomographic images. In this instance, gold is more likely than iodine to absorb photons below 33 keV, however, this range makes little contribution to a recorded image. Above 33 keV, the k-edge of iodine, both materials have
3. X-ray Energy analysis

nearly identical attenuation characteristics. Figure 3.4 shows the anticipated attenuation of a spectrum at 110 kVp. In this instance, more than half of the transmitted photons occur above 80.7 keV, the value of gold’s k-edge. The expected percent difference in contrast due to attenuation of measured spectra is shown as a dashed line in Figure 3.5. This data has been compared to the percent increase in CNR of AuNPs relative to iopromide for phantom images. Note the general trough shape in percent improvement. At low tube potentials (<60 kVp) gold shows greater contrast enhancement. At moderate energies, both CM samples have similar overall attenuation characteristics, while at high diagnostic tube potentials (>100kVp) AuNPs display better performance.

Figure 3.3: Spectrum at 80 kVp showing calculated attenuation by experimental contrast media (gold and iodine). Attenuated photons are plotted as signal (gray dotted lines)
3. X-ray Energy analysis

Figure 3.4: Spectrum at 110 kVp showing calculated attenuation by experimental contrast media (gold and iodine). Attenuated photons are plotted as signal (gray dotted lines)
3. X-ray Energy analysis

Figure 3.5: Percentage difference in calculated attenuation from measured energy spectra (described as "signal") between gold and iodine. These results have been compared to the percentage variation in contrast enhancement from image studies given in Table 2.1 & Table 2.2.

CNR data from images were similar to estimated contrast based on transmitted energy spectra at tube potentials below 120 kVp. Data are summarised in Table 3.1. At 70 and 80 kVp, the estimates are within 4% of the measured values in projection images. When compared with the measured change in contrast in CT, the theoretical model is less accurate, but it does follow the same trend as the CT data. The calculated attenuation correlates closely with measured contrast enhancement at 80 kVp, but at 100 kVp enhancement is overestimated by 25% and at 120 kVp it increases to 51%. We attribute this to three factors. The energy spectra used for calculations were obtained from projection x-ray equipment due
3. X-ray Energy analysis

to the structural limitation of tomographic machines. The model relies on uniform detection efficiency at the image receptors for photons of different energies. It assumes that increases in attenuation produces a linear response in HU density. It is also important to distinguish between iodinated CM occurring in a liquid state, while AuNPs exist in liquid as a colloidal suspension of small crystalline clusters. This means that iodine atoms have a more homogenous distribution within the volume. The effect of inhomogeneity on this nano-scale probability of x-ray photon interaction has not been previously explored in detail, but some attention is given in chapters 4 & 5.

<table>
<thead>
<tr>
<th>Tube Potential (kVp)</th>
<th>Projection (CR)</th>
<th>Computed Tomography</th>
<th>Estimated from X-ray Spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>88.00</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>50</td>
<td>33.27</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>60</td>
<td>22.36</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>70</td>
<td>19.02</td>
<td>--</td>
<td>18.28</td>
</tr>
<tr>
<td>80</td>
<td>13.23</td>
<td>6.80</td>
<td>10.33</td>
</tr>
<tr>
<td>90</td>
<td>--</td>
<td>--</td>
<td>38.25</td>
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<tr>
<td>100</td>
<td>--</td>
<td>48.52</td>
<td>73.75</td>
</tr>
<tr>
<td>110</td>
<td>--</td>
<td>--</td>
<td>102.62</td>
</tr>
<tr>
<td>120</td>
<td>--</td>
<td>78.04</td>
<td>128.91</td>
</tr>
<tr>
<td>140</td>
<td>--</td>
<td>112.71</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 3.1: Calculated percentage increase in attenuation using gold nanoparticles relative to iodinated contrast media. Values are based on calculated attenuation of measured X-ray energy spectra with varying tube potential. Results from contrast analysis in projection and CT images (from chapter 2) are shown for comparison.

One important consideration is the hardening effect of soft tissue on the x-ray beam spectrum. Spectrum measurements indicate that after passing through a patient’s body, the majority of photons collected at the image receptor are above 60 keV in energy. The optimal range for iodine atoms to attenuate x-rays occurs below this threshold. Gold may be better suited for applications that require imaging through the abdomen or upper torso because its
3. X-ray Energy analysis

K-edge value will have a much more pronounced impact on photons available at the image receptor, those above 60 keV.

3.3.2 Limitations of Energy resolution and Dose Rate with CdTe Detector

A cadmium telluride detector offers a number of valuable tools for analysing an X-ray imaging system according to the tubes output spectrum, but the apparatus does suffer from technical limitations. The detector’s resolution is very good when describing the peaks of a low-activity radionuclide source. The gamma ray spectrum of a $^{241}$Am source is shown in Figure 3.6. In this case, only simple collimation is required to achieve an optimal count rate. This spectrum represents a steady input of between 650 and 700 gamma rays per second at the detection surface.

Figure 3.6: Measured spectrum of Gamma Rays emitted by 241-Am source during calibration. Count rate is 684 photons per second yielding very high energy resolution. The peak at 59.5 keV in this spectrum has a full width half maximum of 1.12 keV.
3. X-ray Energy analysis

When the count rate is considerably greater than the example shown in Figure 3.6, our experiments indicate that the energy resolution decreases significantly. Figure 3.7 shows X-ray energy spectra captured after travelling only through a 2 mm thickness of experimental contrast media, gold nanoparticles or iopromide, at a concentration of 200 mg/mL. The X-ray beam can readily transmit through this limited amount of attenuating material resulting in a very high dose rate, particularly at a tube potential as high as 130 kVp. The spectra shown in Figure 3.7 are compiled from three separate 4 second exposures for each CM sample. Each of the 6 exposures yielded between 520 and 580 thousand counts, to describe the relative count rate of 140,000 photons per second (approximately 200 times greater than with our calibration sources).
Figure 3.7: Energy spectra of X-rays transmitted through gold and iodine CM samples for a diagnostic X-ray tube operating at 130 kVp. Shifts in transmission at each element’s K-edge show limited resolution. The full-width half-maximum values of the tungsten characteristic peaks are between 4.1 and 4.8 keV. Energy spectrum measurements are the average of

The tungsten characteristic peaks at 59 and 67 keV are visible, but the full-width half maximum values range between 4.1 and 4.8 keV. That value is four times wider than the peaks for a slow dose rate radioactive source. The K-edge values of iodine and gold have been labelled. The increase in photoelectric absorption for photons exceeding 33 keV is evident in the iodine sample. The edge, however, is spread over a range of 7 keV rather than appearing as sharp decline in X-ray transmission at the 33.2 keV threshold. For that reason, we have chosen to calculate contrast enhancement based on attenuation by known attenuation coefficients rather than by measurement of transmission through CM samples.
The effects of photon-counting pile-up are also clear near the upper energies of the measured spectra. At 130 kVp, the maximum energy of X-rays emitted by the tube should be 130 keV. Those counts at higher energies represent instances when the energy of two photons is deposited within the smallest timeframe for a single X-ray pulse. That is, a 65 keV photon and a 90 keV photon are recorded as a single pulse with 155 keV of energy.

Figure 3.8: X-ray energy spectrum at 130 kVp through AuNP sample with estimated area of photons counted due to signal pile-up shown. Maximum photon energy is expected at 130 keV. Pile-up counts—the result of combining the energy of two discrete X-rays a single pulse—are predominantly of high-energy.
3. X-ray Energy analysis

3.3.3 Calculated absorption by other prospective nanoparticle elements

Several other species of nanoparticles have been the subject of experimentation as the basis for possible contrast agents. Some of these have been discussed in 1.5.1 “Properties of Nanoparticles”. In this section, the fractional attenuation of X-ray beams is calculated for several materials that might be considered for use as a nanoparticle CM. This offers some insight into the possibility of tuning the particle core material to a desired energy range in the hope of maximising attenuation.

The materials have been chosen based on possible viability or appearance in published articles. Iodinated nanoparticles have been described by Galperin et al. (63). Balan et al. describe a technique for the synthesis of bismuth-cored nanoparticles (86), while Rabin et al. have implemented such a species for enhancement of computed tomography in a mouse model (68). Bismuth triiodide nanoparticles (BiI$_3$) have been described (133). These particles would have the advantage of combining two radiopaque elements, each with its own distinct K-edge peak allowing more-uniform attenuation over a range of different tube potentials. Dendrimer-encapsulated platinum nanoparticles have been described by Xu et al. (134). Gadolinium-based MRI contrast agents are also radiopaque and have been explored for use as an X-ray contrast agent. Moreover, Oyewumi et al. describe the use of Gd-based nanoparticles biologically as a dose-enhancement agent in neutron-capture therapy (135).

The same process of calculating attenuation described in this chapter’s methodology was utilised. Materials are described as the addition of 1 cm thickness at a concentration of 10 mg/cm$^3$ and attenuation data is obtained from Berger et al. (9). The fraction of attenuated X-rays for beams at 70 to 120 kVp (Figure 3.2) are summarised below.
3. X-ray Energy analysis

Figure 3.9: Mass attenuation coefficients for a variety of heavy elements. These materials have all been considered as core materials for radiopaque CM or have readily documented colloid synthesis protocols.

<table>
<thead>
<tr>
<th>Tube Potential (kVp)</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine</td>
<td>0.0972</td>
<td>0.0768</td>
<td>0.0576</td>
<td>0.0486</td>
<td>0.0412</td>
<td>0.0356</td>
</tr>
<tr>
<td>Gold</td>
<td>0.0959</td>
<td>0.0642</td>
<td>0.0566</td>
<td>0.0598</td>
<td>0.0591</td>
<td>0.0579</td>
</tr>
<tr>
<td>Platinum</td>
<td>0.0927</td>
<td>0.0648</td>
<td>0.0614</td>
<td>0.0628</td>
<td>0.0608</td>
<td>0.0587</td>
</tr>
<tr>
<td>Bismuth</td>
<td>0.1060</td>
<td>0.0717</td>
<td>0.0515</td>
<td>0.0497</td>
<td>0.0510</td>
<td>0.0524</td>
</tr>
<tr>
<td>Bismuth Triiodide</td>
<td>0.1023</td>
<td>0.0759</td>
<td>0.0560</td>
<td>0.0494</td>
<td>0.0451</td>
<td>0.0420</td>
</tr>
<tr>
<td>Gadolinium</td>
<td>0.1143</td>
<td>0.0958</td>
<td>0.0761</td>
<td>0.0665</td>
<td>0.0579</td>
<td>0.0504</td>
</tr>
</tbody>
</table>

Table 3.2: Fractional absorption values for perspective radiopaque nanoparticle core elements at a variety of tube potentials according to measured X-ray energy spectra.
Figure 3.10: Calculated fractional attenuation of measured X-ray spectra at potentials between 70 and 120 kVp for prospective nanoparticle materials. Area mass for each element or compound is 10 mg per cm\(^2\) (equivalent to a 1 mm vessel at a concentration of 100 mg radiopaque element(s) per mL).

Figure 3.10 shows the fractional attenuation of the measured energy spectra by the considered materials. There is a general trend indicating that heavier elements are better-suited toward high-kVp applications. The greatest fractional attenuation at 120 kVp is seen with gold (Z=79) and platinum (Z=78). These materials attenuated 5.8 and 5.9% of the incident X-ray beam, respectively. That indicates that gold is attenuating 1.6 times as many photons as iodine at 120 kVp. Interestingly, Gadolinium—a common basis for MRI contrast media—displays the highest fractional attenuation of all materials. At 70 kVp, Gd is shown to...
3. X-ray Energy analysis

attenuate 11.4% X-ray photons. The combination of two radiopaque elements in bismuth triiodide had little effect. Though bismuth has a comparatively high K-edge (90.5 keV), only a slight increase in fractional attenuation was shown at high tube potentials.

In general, gold shows comparable X-ray absorption to other heavy elements. Given that gold nanoparticles can be easily synthesised and are relatively biocompatible, the element is a good basis for designing novel radiographic contrast media.

3.4 Conclusions

Results from these experiments provide support for image data reported in chapter 2. Contrast enhancement is shown to be potential-dependent and can be attributed to shifting the transmitted energy spectrum into a range exceeding the absorbing element’s K-edge. In the case of gold, an optimal energy spectrum can be obtained by using tube potentials in the range of 120 kVp or higher. The technique of measuring X-ray photons by CdTe detector offers a robust method for characterising the energy fluence of an X-ray beam. For elements with comparatively high K-edge values, estimating attenuation based on a measured or calculated energy spectrum is more accurate than characterising a beam’s energy by half-value thickness. Comparing the absorption of measured energy spectra between high-Z elements, it is indicated that gold will exhibit comparable or improved enhancement at most tube potentials. Particularly when considering tube potentials in the range of CT imaging (120 kVp and higher), only platinum displayed comparable absorption efficiency.
4 Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

4.1 Introduction

X-ray absorption spectroscopy (XAS) is a tool that allows one to very precisely quantify a material’s absorption coefficient ($\mu$). In XAS, high-intensity synchrotron radiation passes through a silicon crystal monochromators which filters all but a desired photon wavelength from the beam’s path. Adjustments to angle of the monochromating crystal alters the transmitted wavelength according to Braggs law, allowing for the computation of radiation attenuation by a sample with very high energy resolution. This technique is particularly valuable when the photon beam is tuned to the range near the K- or L-edge of an element-of-interest. In practice, it has been shown that variations in bonding have a small, but specifically measurable effect on the amplitude of X-ray absorption in this range of energies. This is related to the probability of the ejected photo-electron being scattered by neighbouring atoms and returning to its original nucleus. We consider this of interest because gold nanoparticles are documented to have markedly different bonding from bulk gold or free ions (77, 80, 81, 136, 137).

This process of photoelectric absorption is the dominant form of attenuation for high-Z elements such as gold and iodine when considering the majority of the diagnostic X-ray spectrum. Measurable changes in X-ray attenuation may be worth consideration when quantifying contrast enhancement by a material such as gold nanoparticles. In this experiment, we utilise XAS to examine the $L_3$-edge of gold nanoparticles. This is compared against the absorption by an Au reference foil and theoretical data based on documented Au crystallographic structure (138).
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

As a second consideration, we were presented the opportunity to expose a portion of our samples to neutron radiation with the hope of examining any structural changes that might occur from Au neutron capture and subsequent decay. In such a fashion, gold nanoparticles may provide some use in neutron detection, where a captured \( n^0 \) induces decay and a break in Au-Au bonding. Irregularities in bonding characteristics may lead to particles splitting into smaller sizes, a phenomenon that would be detectible by common ultraviolet-visible light absorption (139). It is also reported that secondary neutrons are a by-product of photonuclear reaction in 18 MV radiotherapy treatments (140, 141). Radiotherapy is often undertaken in conjunction with kilovoltage-range X-ray images to aid in registration of target tissue volumes (142, 143). If gold nanoparticles are to be considered as contrast media in this low-energy image set, there is a possibility of Au neutron capture and subsequent formation of mercury atoms in the patient. The formation of activated \(^{198}\text{Au}\) nanoparticles have been described by Kannan et al. and Khan et al. (144, 145). In those works, the authors suggested that \(^{198}\text{AuNPs}\) could be considered for use as localised nanobrachytherapy devices for radiation oncology.

This experiment was also aimed at identifying any structural effects that might be induced on nanoparticles from irradiation by neutrons or high-energy photons. The delicate structure of gold nanoparticles compared to bulk structure has been shown to be susceptible to bond rearrangement merely from exposure to the electron beams in transmission electron microscopy. Through collaboration with the Australian synchrotron we sought to identify any structural effects that could be produced by irradiation by other subatomic particles. Of particular interest was neutron-activation of gold atoms. Capture of high-energy neutrons by gold atoms leads to the formation of species of 198-Gold isotope \((^{197}\text{Au} \rightarrow ^{198}\text{Au})\) (146). That isotope decays through alpha emission to form \(^{198}\text{Hg}\) (\( t_{1/2} = 2.7\text{d} \)) (147); effectively producing mercury point defects in the gold nanoparticle structure.
The effect of gold to mercury conversion manifest in the form of changes in chemical bonding in the gold nanoparticle structure. For example, bulk gold takes the form of a face-centered cubic crystal arrangement while mercury atoms arrange in a rhombohedral fashion. Mercury, as a solid, forms much weaker bonds and has a considerably lower density, accordingly (13.53 vs. 19.32 g/cm³) (148). Mercury lies in group IIB of the periodic table and shares its reaction and atomic characteristics with Cadmium and Zinc, while gold is in group IB. There are very few compounds that contain Au-Hg bonds. The notable exception is Au₂Hg, which may form as a hexagonal crystal. In fact, liquid mercury dissolves solid gold to form a two-phase amalgam of Hg(l) and Au₂Hg (148, 149).

Au-Au bonding displays a signal in XAFS (from XAS/XANES), which –after Fourier transformation– shows a representative peak at 2.88 Å (138). This is suppressed in gold nanoparticles due to limited amount of regular crystalline bonding. This has been reported in nanoparticles 1-4 nm in size. XANES also shows striking variations in particles based on their sample preparation and treatment methods, representative of different capping agents and the nature of the material’s bonding to catalytic substrate (77).

4.2 Materials and Methods

4.2.1 Preparation of neutron-irradiated particles

Gold nanoparticle samples were loaded into small glass vials. Liquid samples contained 50 µL AuNP suspension at concentration of 200 mg Au per mL. AuNP powder samples contained 128 mg gold nanoparticle powder. Vials were separated between control (non-irradiated), photon-irradiated, and neutron-irradiated species. Neutron exposure was accomplished by placing samples in proximity of a 241-Americum/Beryllium radionuclide source in conjunction with collaborators at ARPANSA (the Australian Radiation Protection and Nuclear Safety Agency). Samples were placed at a distance of 3 centimetres from the
centre of the source (shown in Figure 4.1). 1 liquid sample and 1 powder sample added at each irradiation time-point to provide staggered neutron radiation dosage (Table 4.1). The energy spectrum of emitted neutron radiation from a representative Americium/Beryllium Source is shown in Figure 4.2 (150). The absorption cross-section values for capture of thermal neutrons by $^{197}$Au (in barns) are shown in Figure 4.3 (146).

![Figure 4.1: Gold Nanoparticle samples being exposed to neutron radiation from 10 Ci $^{241}$Am/Be source](image-url)
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

Figure 4.2: Energy spectrum of emitted neutrons from decay of $^{241}\text{Am/Be}$ radionuclide source. Recreated from data reported by Edgar A. Lorch (150)

Neutron-capture cross-section values for Au radionuclide production:

$^{197}\text{Au}$ to $^{198}\text{Au}$
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

Figure 4.3: Tabulated cross-section values for radionuclide production component of interaction between Au and neutrons of various energies (146).

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Start time of Neutron Activation</th>
<th>Time</th>
<th>End Date of Activation</th>
<th>Exposure Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19/03/2009 14:30</td>
<td>23/03/2009</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>15/03/2009 14:30</td>
<td>23/03/2009</td>
<td>192</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10/03/2009 14:30</td>
<td>23/03/2009</td>
<td>312</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5/03/2009 14:30</td>
<td>23/03/2009</td>
<td>432</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Exposure timepoints for the 4 neutron-irradiated sample sets (1 liquid & 1 powder sample per set)

4.2.2 Irradiation of samples by Linear accelerator

Photon-irradiated samples were exposed to 18 MV radiation beam with a Varian Clinac® 2100C clinical radiation therapy linear accelerator (Varian Medical Systems Inc., Palo Alto, CA, USA). This energy is widely used for clinical applications and is shown to have a small probability of inducing photonuclear reaction to create secondary neutrons (140, 141, 151). Sample vials were placed into 8 cm diameter cylinder filled with water to act as dosage build-up region. A diagram of the geometric orientation is shown in Figure 4.4.
4.2.3 X-ray Absorption Spectroscopy

X-ray absorption spectroscopy was utilised to quantify the linear attenuation coefficient of gold nanoparticles with respect to bulk Au. Further analysis of measured absorption data was used for structural analysis with the aim of identifying evidence of $^{197}$Au to $^{198}$Hg neutron capture and decay.

Gold nanoparticles were prepared for XAS by first being placed into aluminium sample holders. The sample holders contained an opening of either rectangular (5 mm x 2 mm) or circular (13.3 mm) shape and a cross-sectional thickness of 1 millimetre. Nanoparticle samples were loaded into the vacant volume of the sample holders and sealed on either side with radiolucent Kapton tape (see Figure 4.5). Liquid samples were prepared at concentrations of 100 & 200 mg Au per millilitre. Powder samples were prepared on-site at the Australian synchrotron. 9 milligrams Au nanopowder was mixed with 200 mg boron.
nitride binding agent and compressed into a uniform 1 mm thick, circular pellet. All samples were prepared to achieve an optimal $L_3$-edge shift ($\Delta \mu$) of $\sim$1.

![Figure 4.5: Gold Nanoparticle samples loaded into sample holders for XAS measurements. Samples are contained in aluminium holders enclosed on either side by radiolucent Kapton tape.](image)

Samples were measured on the X-ray absorption spectroscopy beamline of the Australian synchrotron on 16 & 17 April, 2009. This beamline consisted of an adjustable, highly-monochromatic narrow X-ray source, two experimental sample holders, and three ionisation chambers to record beam intensity (initial, after attenuation by sample 1, after attenuation by sample 2). Figure 4.6 shows an image of the beamline during irradiation measurements.

X-ray absorption spectroscopy scans were performed by measuring the X-ray attenuation by experimental samples with varying X-ray wavelength in the range of the Au $L_3$-edge (11,919 eV). The $\mu$ values were measured at varying intervals corresponding to the pre-edge, near-edge, and k-space ranges to optimise scan efficiency. Raw data were then analysed using Matthew Newville’s IFEFFIT extended X-ray absorption fine-structure software packages: Athena and Artemis (152-155). After normalisation and background subtraction in Athena, the atomic radial distribution was determined by Fourier transform to identify any evidence of changes in common bond distances and packing regularity. Data
were imported into Athena for FEFF analysis. Nanoparticle samples (both irradiated and control) were compared to measurements from bulk reference foil and Au crystallographic structure based on measurements by Suh et al. (138) in conjunction with the ATOMS code (154). Theoretical radial distribution function (RDF) was determined based on the contribution of single- and double- scattering events between neighbouring atoms considered within a radius of 5.5 Å.

![Figure 4.6: X-ray Absorption Spectroscopy beamline at the Australian Synchrotron. Beam direction shown right to left in photograph. Three ionisation chambers measure incident beam intensity and transmission after passing through Cryostat, cold sample (right middle) and reference sample holder (left middle)](image)

**4.3 Results and Discussion**

**4.3.1 Estimated Radionuclide conversion**

The absorption of neutrons to produce a radionuclide isotope can be described as a function of neutron flux and particle absorption cross-section. This can be given as:
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

\[ R = \sigma \phi N V \]  

(4-1)

where \( R \) is the rate of neutron interactions per \( \text{cm}^2 \) of target area, \( \sigma \) is the microscopic cross section of interaction (in barns, \( 1 \text{ b} = 1 \times 10^{-24} \text{ cm}^2 \)), \( \Phi \) (\( n^0 \) flux) is the number of neutrons crossing a unit surface area (1 \( \text{cm}^2 \)) per second, \( N^*V \) is equal to the number of nuclei per \( \text{cm}^2 \) of target area (156). It is important to note that after undergoing conversion, nuclei are no longer available to absorb neutrons by the same process. Therefore, the rate of neutron-capture to form the \(^{198}\text{Au}\) isotope decreases over time as the number of available nuclei dwindles. In this case, however, the quantity of Au atoms that undergo conversion is very small relative to number of non-activated Au isotopes. We have considered the effects of rate reduction to be negligible.

For the purposes of this experiment, we are concerned with the fraction of gold atoms that undergo activation and resultant decay. That fraction can be derived as

\[ F = \frac{R^*t}{NV} = \frac{(\sigma \phi N V)t}{NV} = \sigma \phi t \]  

(4-2)

where \( F \) is the fraction of gold nuclei activated to \(^{198}\text{Au}\) (number of converted atoms divided by total quantity of Au atoms), \( \sigma \) is the microscopic cross section of interaction, \( \Phi \) is the number of neutrons crossing a unit surface area (1 \( \text{cm}^2 \)) per second, and \( t \) is the time of exposure in seconds. Because the decay half-life for \(^{198}\text{Au}\) to \(^{198}\text{Hg}\) is considerably shorter than the neutron exposure time we will assume that nearly all activated Au atoms (reaction events) result in the conversion to mercury atoms.

The cross-section of interaction, \( \sigma \), is dependent on neutron speed—or energy—as well as the type of atom. Likewise, the \(^{241}\text{Am/Be}\) source emits a spectrum of different neutron energies. We must define the flux and nuclear cross section values as functions of energy, \( \Phi(E) \), and the nuclear activation cross section of \(^{197}\text{Au}\) as the function: \( \sigma(E) \) where \( E \) is the kinetic energy of incident neutrons. The values of the cross-section for this specific interaction have been tabulated in the ENDF/B-VI report by the National Institute of
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

Standards and Technology and are shown in Figure 4.3 (146). Only the cross-section for the $^{197}$Au isotope is considered because its abundance is nearly 100 percent; no other isotopes of Au appear naturally (157). The function of neutron flux according to energy, $\phi(E)$, is specific to the decaying radioisotope. It can be defined by the energy spectrum reported in Figure 4.2 (150). The flux is dependent also on the level of activity of the neutron source. In the case of the 10 Ci AmBe radioisotope, emission is designated at $2.6 \times 10^7$ neutrons per second. In the absorption equation defined above, flux is given as a rate of neutron emission per unit area. The total decay must be corrected for the surface area of a sphere with a radius of 3 centimetres according to the experimental sample distance during exposure (total emission/113.10 cm$^2$).

The absorption equation must be solved for each discrete energy level and combined with the function for neutron flux based on the source energy spectrum. In this case we find:

$$ F = t \sum_{E=0}^{11\text{MeV}} \sigma(E) \phi(E) $$

The above equation yields a fractional $n^0$ absorption rate for $^{197}$Au from a $^{241}$AmBe source at 3 cm of $3.7607 \times 10^{-21}$ events per Au atom per second (a tabulated summary is shown in appendix 10.1). The fraction of Au atoms converted to Hg per sample is shown below in Table 4.2.

<table>
<thead>
<tr>
<th>AuNP Sample #</th>
<th>Exposure Time (seconds)</th>
<th>Fraction of $^{197}$Au atoms decayed to $^{198}$Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4560E+05</td>
<td>1.2997E-15</td>
</tr>
<tr>
<td>2</td>
<td>6.9120E+05</td>
<td>2.5994E-15</td>
</tr>
<tr>
<td>3</td>
<td>1.1232E+06</td>
<td>4.2240E-15</td>
</tr>
<tr>
<td>4</td>
<td>1.5552E+06</td>
<td>5.8487E-15</td>
</tr>
</tbody>
</table>

Table 4.2: Summary of fractional $^{197}$Au to $^{198}$Hg conversion for neutron-irradiated gold nanoparticle samples
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

It is apparent that the quantity of Hg conversion is very small relative to the overall mass of Au. Even in the sample that had been exposed to the source at a distance of 3 cm (1 cm from its surface) for 18 consecutive days, we have calculated that only 1 atom out of every 170,980,000,000,000 is converted to mercury. In a 100 mg sample, that equates to approximately 1.8 million $^{198}$Hg atoms, but that is just a scant fraction compared to the overall composition. In future experimentation to identify the effects of Hg defects in AuNP structure, it would be advisable to utilise a high-flux neutron source such as a nuclear reactor to efficiently activate a larger fraction of gold material for analysis.
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

4.3.2 X-ray Absorption Spectroscopy Measurements

Here we present the normalised $\mu$ values according to our XAS experimental measurements. Figure 4.7 shows the edge shift ($\Delta \mu$) normalised to a value of 1 after background subtraction. There was some difficulty obtaining high-quality absorption measurements from the liquid samples. Gold nanoparticle concentration was found to be dramatically lower than described by the supplier’s specifications (approximately 20 mg/cm$^3$ rather than the claimed 200 mg/cm$^3$ based on the measured edge shift of $\Delta \mu=0.1$). There was also visible inhomogeneity in the colloid which was attributed to particle aggregation in the time following sample preparation. For viable XAS measurements, experimental samples must be highly uniform in density to account for small displacements in beam position (wobbling) during XAS scans. For these reasons, the amplitude of the measured $\mu$ value fluctuates slightly in the liquid nanoparticles over the measured energy range; seen as statistical noise. The random frequencies imparted by the noise prevented further structural analysis of liquid samples by Fourier transform.
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

Figure 4.7: Normalised $\mu$ values obtained from XAS beamline measurements. The results of liquid samples measured at room temperature (maroon) and in the cryostat at 4° K have been plotted (pink). Neutron-irradiated, photon-irradiated, and non-irradiated (control) gold nanopowder samples are plotted as purple, yellow, and green, respectively. For comparison, the measured XAS spectrum of a gold reference foil is plotted in blue and red.

Each of the nanoparticle samples has been measured until one satisfactory (noise-free) edge measurement was completed. Two scans of the Au reference foil (blue and red in Figure 4.7) show that measured absorption is highly uniform for each sample between scans. Repeat measurements were not required as these only improve analysis by removal of statistical noise before transform. Experimentally, that had already been accounted for through the use of extended scan times (maximising the statistical sampling for measurements of photon absorption at each X-ray energy level in the scan).

Gold nanopowder samples offered more-robust data for structural evaluation. The powder was readily mixed with binding agent to form a uniform sample cross-section to the beam line. In Figure 4.7, there is a visible discrepancy between the measured $\mu$ values for the powder samples (shown as green, purple, and yellow curves) and the gold reference foil (blue and red). The amplitude of the edge in the powdered samples shows a more-rapid increase in X-ray absorption immediately at the L$_3$-edge, 11919 eV, and continuing up to 11946 eV. This is shown in better detail in Figure 4.8 where only foil and powder samples are plotted. Though we see a variation between nanoparticles and bulk gold (foil), there does not appear to be any discernible variation in absorption between the irradiated by n$^0$ nanopowder samples and the non-irradiated control.
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

Further analysis of XAS data by Fourier transform is shown in Figure 4.9. This chart represents the radial distribution function as determined by FEFF analysis in Artemis. All samples show a common peak in the region of 2.8 Å, indicative of the expected nearest-neighbour Au-Au bonding distance in gold solid. The amplitude of the 2.8 Å peak is greatest in the reference foil samples. The edge shift measured for the reference foils (Δµ = 4.9) was greater than any of the nanoparticle samples (Δµ ≈ 2.1, 1.1, 0.2, 0.1, see Table 4.3). In this case, the greater edge shift provided data with improved energy resolution (less noise) during the scan. We attribute the greater amplitude of the peak at 2.8 Å to a reduction in noise in that dataset. Further evidence for this is given in that two scans of the same reference foil produced different peak amplitudes. The position of the peak (at 2.8 Å) did not change, however. That is the case regardless of the sample type, whether reference foil or colloidal or powder nanoparticles.

Figure 4.8: Measured µ values for gold nanopowder samples and Au reference foil.
Figure 4.9: Backward Fourier transform of XAS data to R-space. Magnitude of the peaks in this chart represent the correlation between frequency data from $\mu$ measurements and atomic radius in Angstroms (X-axis). Liquid samples show many, indiscriminate peaks due to low absorption in samples experimentally.

Comparison to crystallographic data allowed discrimination of radial distribution function peaks based on electron scattering path. Figure 4.10 shows the amplitude of radial distribution peaks based on the three primary single-scattering paths (detailed summary is given in Appendix 10.2 Au ATOMS/FEFF Input Data). The peak shown at 2.879 Å represents a scattering event in which photoelectron ejected by the absorbing atom is scattered by the closest neighbouring atom (2.879 Å away). Paths 2 and 5 relate to scattering events with other common atomic distances in the crystal structure.
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

Figure 4.10: Calculated amplitude of the radial distribution function in R-space using ATOMS. This includes single- and double- scattering events based on Au crystallographic data from Suh et al. (138). The electron scattering path lengths are 2.8793, 4.0720, and 4.9872 Å for paths 1, 2, and 5, respectively.

Analysing measured L\(_{3}\)-edge shifts for gold nanopowder samples, we do not find evidence of structural abnormalities in either photon- or neutron-irradiated species. R-space Fourier transformations of XAS data for non-irradiated (control) sample is shown in Figure 4.11. Data have been plotted against the theoretical RDF peaks for bulk gold. We do not find any evidence of significant changes in Au-Au bond-length. Data for gold nanopowder irradiated with highest neutron dose (432 hours) is shown in Figure 4.12. There is no appreciable variation between this sample and the control or the bulk Au structure. Likewise, we do not find any measurable evidence of neutron capture in the nanoparticle samples irradiated by 18 MV linear accelerator. According to data by Vega-Carillo et al., the secondary neutron spectrum at 18 MV peaks at 1 MeV, well below the energy required for activation of \(^{198}\)Au (151).
Figure 4.11: Radial distribution function for non-irradiated gold nanoparticle sample measured by XAS. R-space plot from measured data is shown in blue while FEFF fit based on tabulated Au crystallographic data is shown in red. R-factor of the fit is acceptably low at 0.0144.
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

Figure 4.12: Radial distribution function based on XAS measurement of gold nanoparticles irradiated with highest tested dose of neutron radiation. R-space plot based on measured data is shown in blue while theoretical data is plotted in red. Fit is very close (R-factor = 0.0170). There is no evidence of radial contraction or the presence of irregular bond distance due to mercury formation.

A summary of the analysed datasets is presented in Table 4.3. XAS measurements for nanoparticle samples are compared by goodness-of-fit test against theoretical FEFF data for Au crystal. Variance between Fourier transforms of theoretical and measured transmission data are compared to identify any significant discrepancies. The statistical results of fitting theoretical data to XAS measurements are given according to R-factor, $\chi^2$, and reduced $\chi^2$. Nanopowder samples show closest fit to theoretical data (reduced $\chi^2 < 13$ for all samples). Correspondingly, the R-factor values are very low ($< 0.02$), indicating minimal misfit between measured and bulk data.
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

<table>
<thead>
<tr>
<th>Sample</th>
<th>Edge Step (Δμ)</th>
<th>R-factor</th>
<th>Reduced χ² value</th>
<th>χ² Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Foil 1</td>
<td>4.898</td>
<td>0.0036</td>
<td>59.14</td>
<td>794.6</td>
</tr>
<tr>
<td>Reference Foil 2</td>
<td>4.877</td>
<td>0.0062</td>
<td>85.18</td>
<td>1533.3</td>
</tr>
<tr>
<td>Powder Control (non-irradiated)</td>
<td>2.112</td>
<td>0.0144</td>
<td>4.92</td>
<td>78.4</td>
</tr>
<tr>
<td>Powder - High Neutron Dose</td>
<td>2.056</td>
<td>0.0170</td>
<td>6.30</td>
<td>100.4</td>
</tr>
<tr>
<td>Powder - Photon Irradiated</td>
<td>1.149</td>
<td>0.0057</td>
<td>12.25</td>
<td>195.3</td>
</tr>
<tr>
<td>Liquid - High Neutron Dose (Measured in Cryostat)</td>
<td>0.233</td>
<td>0.6938</td>
<td>70.44</td>
<td>1298.8</td>
</tr>
<tr>
<td>Liquid - High Neutron Dose (Measured at Room Temperature)</td>
<td>0.097</td>
<td>0.1312</td>
<td>332.85</td>
<td>6136.9</td>
</tr>
</tbody>
</table>

Table 4.3: Statistical values of FEFF fits of theoretical Au Crystal data calculated by ATOMS and experimental XAS measurements at Au L₃-edge

4.4 Conclusions

Measurements by X-ray absorption spectroscopy indicated the presence of Au in nanoparticle samples. Neutron irradiation did not elicit any measurable change in X-ray absorption or Au structure in experimental nanoparticle samples. We did, however, see a small variation in X-ray absorption between gold nanoparticles and reference foil. The lack of structural change in Au nanoparticles following n⁰ irradiation is attributed to very small quantities of neutron capture by Au for thermal neutrons from the AmBe source (on the order of 10⁻¹³%). Future experimentation would require the use of a high flux source such as an experimental nuclear reactor. Moreover, neutron capture efficiency can be increased through the use of neutrons with energy between 10 and 20 MeV. Only a fraction of the radiation utilised in this experiment had sufficient energy to yield the formation of ¹⁹⁸Au. Moreover, we find that there is no evidence to suggest that secondary neutrons in 18 MV photon radiotherapy will induce significant neutron capture in gold nanoparticles.
4. Characterisation of Au Nanoparticles by X-ray absorption Spectroscopy

XAS was shown to be an effective technique for measuring the structure of Au based on L$_3$-edge absorption. Nanoparticles displaying a greater structural discrepancy with respect to bulk Au would be preferable to identify augmented bonding due to quantum confinement (preferably below 5 nm in some dimension).
5. Effect of Nanoparticle shape and size on image contrast

5  Effect of Nanoparticle shape and size on image contrast

5.1  Introduction

In recording contrast in a radiographic image, we are concerned with detecting the differential attenuation of generated X-rays as they pass through the patient. Materials that attenuate more photons in the range of emitted X-rays will appear more brightly on the corresponding radiograph. In general, attenuation is dictated by three factors: density, elemental composition, and thickness. It is conventional to consider that an element attenuates photons to the same degree regardless of how it is bound to neighbouring atoms. It has been shown, however, that variations in X-ray attenuation near an element’s K- or L-edge can be indicative of its bonding and distance between nearby nuclei (158). Following photoelectric absorption, the ejected photoelectron may undergo single- or multiple-scattering events with neighbouring atoms. This effect, along with slight fluctuations in the emission of auger and fluorescent photons, can alter the attenuation coefficient of an element relative to the bulk material when considered over a small range of X-ray energies.

The effect of nanoparticle size and capping agent (surfactant) on changes in attenuation coefficient, as relevant to X-ray absorption spectroscopy, has been well documented (77, 80, 137, 159-161). There has not been any investigation of particle size effect on attenuating a broad-spectrum, polychromatic X-ray beam in radiology. The aim of this experiment was to organise several samples of gold nanoparticle suspensions—each with the same concentration by mass of gold atoms— but with a large disparity in mean particle size and surfactant. This would be beneficial to identify whether the variations in attenuation that are evident in XAS could be utilised to improve contrast enhancement in radiographic imaging.
5. Effect of Nanoparticle shape and size on image contrast

5.1.1 Pharmacokinetic Effects of Nanoparticle Size, Shape, and Capping Moiety

The biological distribution of pharmaceutical nanoparticles is determined by a variety of physiological and pharmacological factors (162). Moghimi and Hamad explain that the path of these materials in the body is affected by nanoparticle size, shape, density, rigidity or deformability, and surface characteristics (electric charge, surface density, conformation of synthetic polymers and biological ligands) (163). Such properties affect the way that nanoparticles flow intravenously. They can also affect circulation time and cellular uptake. It has been reported that liposome-capped nanoparticles rapidly coalesce with a coating of blood plasma proteins following injection (164). Chonn et al. reported that high affinity to blood proteins can rapidly reduce circulation time. In order to prolong the clearance half-life, a surfactant should be chosen with low specificity for human blood proteins (164).

Likewise, particle diameter can also dictate clearance kinetics. In order to undergo renal clearance, nanoparticles must be small enough to efficiently exit through the excretory system by glomerular filtration. In this case, the pores allowing transition of materials from one side of the boundary to the other are approximately 4 nm in size (47). Particles with significantly greater diameters should continue to circulate through the blood stream until they are removed by filtration in hepatic cells (as shown in (96)) or they are targeted for phagocytosis by macrophage cells and eventually accumulate in lymphatic tissue. Decreasing excretion rate would also allow more time for CM to flow across a damaged blood-brain barrier, potentially improving visualisation of cerebral malignancies. Tailored particle size might offer tissue specificity depending on suspected pathology. Changing particle excretion or accumulation routes shows promise to specifically improve visualisation of either hepatic carcinomas or cancers of the lymph nodes.
5. Effect of Nanoparticle shape and size on image contrast

5.1.2 Gold Nanoparticle Synthesis

A multitude of synthesis procedures are available to produce clusters of gold nanoparticles, that range in size from less than a dozen to over ten thousand individual atoms. Figure 5.1 shows the cuboctahedral arrangement of gold atoms in Au_{13} and Au_{55} nanoparticle clusters (165, 166). The standard precursor for the production of gold nanoparticles is chloroauric acid (HAuCl₄). Reduction of chloroaurate ions (AuCl₄⁻) causes a displacement of a chlorine atom to form an Au-Au bond. This is effectively the reduction of Au(III) to the form of Au(0) (167). The initial bonding of these ions is known as the nucleation or seeding stage. Afterwards, subsequent reduction of free chloroaurate ions in solution produces further Au-Au bonding which results in an increase in cluster size. This second stage, or growth stage, varies highly depending on the reaction-type. In some cases, growth occurs steadily until all Au(III) precursor has undergone reaction (168). In other instances, nanoparticle growth stops after particles exceed some critical size. In this case, the remaining free gold ions continue to produce new nanoparticle seeds until all free chloroaurate ions have reacted (167). It has also been reported that growth continues even after the Au(III) species has been exhausted. Large particles are often the result of bonding between smaller nanoparticles (167).
5. Effect of Nanoparticle shape and size on image contrast

It is evident that the synthesis route has an impact on particle size and the related percentage make-up of surface atoms. The mechanism of nanoparticle growth—whether it be by bonding of free Au(III) ions or by clustering of smaller particles—is likely to affect how well the metallic nanoparticle core resembles the bonding of a well-ordered bulk crystal. That is, steady growth by bonding to the nanoparticle surface of Au(III) ions produces more regular packing while the accumulation of multiple small clusters is likely to contain some defects. In a structure laden with many defects in bonding, the general electronic behaviour is more likely to resemble that of single atoms than of bulk crystal.

5.2 Materials and Methods

5.2.1 Gold nanoparticle synthesis

Three synthesis techniques have been used to produce gold Nanoparticles with various sizes, shapes, and solubility-types. A fourth sample of gold Nanoparticles was also obtained from an outside supplier for further comparison, albeit with limited understanding of its precise chemical structure. In this fashion, a number of different criteria relating to surface and capping effects on the possible change in contrast enhancement have been evaluated.
5. Effect of Nanoparticle shape and size on image contrast

Concerted effort was placed into these particle syntheses. A wider variety of AuNP samples would have been beneficial, but further efforts into experimental gold nanoparticle synthesis were beyond the resources available for this work. Each type of particles described below was synthesised as a single batch, utilising large reactant volumes to obtain the desired product yield.

5.2.1.1 Hydrophobic Gold Nanoparticles Stabilised by Hexadecylamine

A solution containing 250 mL of $2.5 \times 10^{-4}$ M HAuCl₄ was prepared. Sodium citrate was added to the solution (18.38 mg, final concentration of $2.5 \times 10^{-4}$ M). Sodium Borohydride (NaBH₄ - 24.6 mg) was dissolved in 10 mL of water and while stirring. Upon addition of the reducing agent, the solution quickly changed from yellow to orange-red in colour. Solution was left on magnetic stirrer for one hour to allow the reaction to continue until reactants had been exhausted. After one hour, a dark red suspension of citrate-capped gold Nanoparticles were evident. To this solution, 250 mL chloroform (CHCl₃) with 300 mg hexadecylamine (final concentration $5.0 \times 10^{-3}$ M) was added with continued stirring. This step resulted in capping the nanoparticles with a layer of non-polar hydrocarbon chains that garner the particles with a high level of hydrophobicity. The addition of the chloroform solution produced a rapid phase-transfer of the nanoparticles from the aqueous to organic layer. Within minutes, the aqueous phase had changed from deep red to fully transparent, indicating a nearly complete transfer of nanoparticles into the organic phase. The heavier organic phase was then drained and rotovapped. The dried particles could be easily resuspended in organic solvents; in this case chloroform. Prior to imaging, the samples were again dried and resuspended in toluene to more closely match the density of aqueous solvents used to suspend other nanoparticle samples.
5. Effect of Nanoparticle shape and size on image contrast

5.2.1.2 Gold Nanorods Synthesised by Radiolysis

Hexadecyltrimethylammonium bromide (CTAB - 1.18 g) was mixed in a beaker with 40 mL milliQ water. Solution was heated to 50° C with stirring. After complete dissolution of CTAB in water, 16.4 mg of tetraoctylammonium bromide (TOAB) was added with continued stirring. Immediately afterwards, 3.2 mL of chloroauric acid (2.5*10^{-2} M) was added. Solution was left stirring for one hour. Acetone (860 µL) cyclohexane (660 µL) were added sequentially. Silver nitrate was added (1 * 10^{-2} M, 100 µL). Solution was transferred into vials and exposed to UV light for 4 hours to activate the chemical reaction. After irradiation, solution had changed from yellow to blackish-purple in colour indicating the formation of gold nanorods. The suspension was centrifuged at 5000 rpm for 30 minutes. The precipitate was resuspended in water for imaging.

5.2.1.3 Synthesis of PVA Polymer-stabilised gold nanoparticles

A 1% solution of polyvinyl alcohol (PVA) was prepared by mixing 320 mg PVA with 32 mL deionised water. Solution was heated to dissolve all PVA and left for an excess of 30 minutes to ensure homogeneity. Chloroauric acid (1.0 * 10^{-2} M, 8 mL) was added and solution was transferred into quartz vial. Solution was exposed to ultraviolet light for 4 hours. After irradiation, the sample had changed from yellow to deep red in colour. The suspension was centrifuged at 5000 rpm for 30 minutes. Supernatant was removed leaving a viscous precipitate. The precipitate was resuspended in water and then made ready for imaging.
5. Effect of Nanoparticle shape and size on image contrast

5.2.2 Particle Dilution & Sample Preparation

For imaging, all samples were diluted to the same concentration of 5 milligrams radiopaque element per millilitre. AuNP syntheses were anticipated to yield nearly 100% mass of Au, but in order to account for incomplete reactions and loss of product due to particle aggregation and precipitation from suspension the final concentration was confirmed by Atomic Absorption Spectroscopy (AAS). Fifty microlitre aliquots of gold nanoparticle suspensions were dissolved in 1 mL freshly-prepared aqua regia (1:3 nitric acid and hydrochloric acid). Samples were diluted to a volume of 10 mL with milliQ H₂O (dilution factor 1:200). Concentration was quantified by AAS (AA280FS atomic absorption spectrometer, Varian Inc., Palo Alto, CA, USA). Final sample dilutions were completed in accordance with these values to yield CM samples at concentrations of 5 mg/mL.

For imaging, samples were loaded into glass vials (6 mm inner diameter, 8 mm outer diameter) as shown in Figure 5.3. Each sample was measured out to a volume of 1000 µL (due to low product from synthesis, gold nanorod sample volume was limited to a volume of 700 µL).
5. Effect of Nanoparticle shape and size on image contrast

5.2.3 Gold Nanoparticle Characterisation by Electron Microscopy

Fifty microlitre volumes of Gold nanoparticle suspensions were deposited onto carbon-coated copper grids. Liquid was left to evaporate over night resulting in a thin layer of each AuNP sample. TEM (Transmission Electron Microscope) micrographs were collected on a JEOL JEM 1010 Microscope (JEOL Ltd., Tokyo, Japan) at 100 keV (Figure 5.2).

Figure 5.2: JEOL JEM-1010 transmission electron microscope used to measure gold nanoparticle size and shape
5. Effect of Nanoparticle shape and size on image contrast

Images were recorded digitally. Particle size distributions were measured with ImageJ (National Institutes of Health, USA) according to calibrated scale from electron microscope.

5.2.4 Imaging protocol

Projection images were recorded onto PSP cassettes (Kodak Industrex® GP) and scanned on a Kodak DirectView CR900 scanner (Eastman Kodak Inc., Rochester, NY, USA). All samples were included in each image to allow simultaneous acquisition and prevent any measurement bias due to fluctuations in image noise between exposures and scans (arrangement shown in Figure 5.3). Eight centimetres PMMA scatter material was placed in front of samples to replicate beam hardening and scatter produced by human tissue in vivo (see Figure 5.4). Images recorded at maximum focus-film-distance, 327 cm to maximise uniformity of beam at image receptor by reduction of anode heel effect. CM samples were located at a distance of 250 cm from focal spot (as close to the image receptor as the table would permit). Samples were agitated immediately prior to exposure to minimise suspension inhomogeneity due settling. Three images were recorded at each tube potential: 40, 60, 80, and 100 kVp.

Radiographs were analysed for contrast-to-noise ratios at CM regions. For each sample, two background ROIs were chosen (one left and one right of the sample as appearing in image). One region was chosen in the centre of the CM sample. ROIs consisted of rectangular shape with dimensions of 11 x 33 pixels. Contrast is determined as the mean difference in image gray value between CM ROI and background ROIs. Image noise is measured as mean of the standard deviation in image gray value in each background ROI.
5. Effect of Nanoparticle shape and size on image contrast

Figure 5.3: Experimental CM samples in vials during X-ray imaging. Samples are (left to right): PVA-stabilised gold spheres, gold nanorods, Omnipaque® (iohexol) iodinated CM, 6nm organic-phase spheres, and Hexadecylamine-stabilised spheres.
5. Effect of Nanoparticle shape and size on image contrast

Figure 5.4: Orientation of experimental CM samples, PMMA scatter material, and X-ray tube as used in radiographs

5.3 Results and Discussion

5.3.1 Transmission electron Micrographs

TEM micrographs of experimental gold nanoparticle samples are shown in Figure 5.5, Figure 5.6, Figure 5.7, and Figure 5.8. Measured particle size distributions are inset in each image. Synthesis procedures were successful in producing a wide range of particle sizes. Gold nanospheres range in diameter from 3.8 to 25.0 nanometres while the larger gold nanorods can be described by a mean diameter of 29.8 nm and length of 63.0 nm.
5. Effect of Nanoparticle shape and size on image contrast

Figure 5.5: Gold nanorods synthesised by radiolysis. Mean particle length and diameter are 63.0 and 29.8 nm, respectively
Figure 5.6: Organic-Phase gold nanoparticles stabilised by Hexadecylamine. Mean particle diameter is 3.8 nm
Figure 5.7: Organic-phase gold nanoparticles from outside supplier. Particles are poorly-defined in this micrograph due to small size and low concentration. Mean diameter is 5.8 nm.
5. Effect of Nanoparticle shape and size on image contrast

Figure 5.8: PVA polymer-stabilised gold nanoparticles. Note large dispersion range; mean diameter is 25.0 nm

Size measurements by electron microscopy indicate that particle syntheses yielded batches of nanostructures in disparate size ranges \(~5\) and \(~30\) nm. This range of sizes allows comparison of very small (less than 5 nm) particles against AuNPs with larger dimensions. Based on mean nanoparticle size, it is possible to estimate the percentage of Au surface atoms relative to the structure’s overall mass. Here, we assume particle packing density is uniform and equal that of bulk gold crystal. Generalising particle shape as either simple sphere or
5. Effect of Nanoparticle shape and size on image contrast

cylinder, and assuming that all surface atoms are enclosed in the volume comprised of the last
2.8 Å of the particle’s dimensions (the mean Au-Au bond distance in bulk gold), it is possible
to calculate the percentage of surface atoms in each nanoparticle sample. A graphical
representation of these volumes is given in Figure 5.9. A numerical description is represented
in Table 5.1. Samples with small diameters are shown to have a greater surface component.
3.8 and 5.8 nm samples are estimated to be comprised of 39 and 27 % surface atoms,
respectively. The surface component of the larger nanorod samples is estimated at less than
five percent. Samples with a large quantity of surface atoms are anticipated to exhibit a
greater discrepancy in X-ray absorption due to quantum confinement effects (77).

Figure 5.9: Geometric representation of Au nanorods (left) and spheres (right). The inner
volume, considered to contain fully-coordinated Au atoms, is shown in yellow. The volume
containing disordered surface atoms is shown in orange. The volume containing surface
atoms is represented by the outer 2.8 Å of the particle’s dimensions.
5. Effect of Nanoparticle shape and size on image contrast

<table>
<thead>
<tr>
<th>Nanoparticle Sample</th>
<th>Mean Particle Diameter (nm)</th>
<th>Mean Particle Height (nm)</th>
<th>Particle Volume (nm³)</th>
<th># of Au atoms per particle</th>
<th># of atoms per outer 2.8 Å of surface</th>
<th>Percentage surface atoms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold Nanorods</td>
<td>29.8</td>
<td>63.0</td>
<td>43940</td>
<td>2592883</td>
<td>122232</td>
<td>4.7</td>
</tr>
<tr>
<td>PVA-stabilised nanospheres</td>
<td>25.0</td>
<td>n/a</td>
<td>8181</td>
<td>482768</td>
<td>32650</td>
<td>6.8</td>
</tr>
<tr>
<td>HDA-stabilised nanospheres</td>
<td>3.8</td>
<td>n/a</td>
<td>29</td>
<td>1695</td>
<td>661</td>
<td>39.0</td>
</tr>
<tr>
<td>Organic-phase nanospheres</td>
<td>5.8</td>
<td>n/a</td>
<td>102</td>
<td>6028</td>
<td>1626</td>
<td>27.0</td>
</tr>
</tbody>
</table>

Table 5.1: Analysis of surface component for gold nanoparticle samples based on size distributions from TEM micrographs

5.3.2 Size effects on net attenuation – contrast enhancement

Images of contrast media are represented in Figure 5.10. The four experimental nanoparticle samples (3.8, 5.8, & 25 nm spheres and 63x29 nm nanorods) are shown beside an iohexol control. There is little visual variation between CM regions. The walls of the glass vials can be seen around the outer millimetre of the samples. Attenuation by colloidal and iodinated CM is still measurably greater than a control H₂O sample (see Figure 5.11).
5. Effect of Nanoparticle shape and size on image contrast

Figure 5.10: Radiographic images of experimental CM samples. Enhancement is highly uniform across samples. Increasing tube potential results in decreased contrast enhancement.

![Radiographic images of experimental CM samples](image)

Measured CNR Values vs. Potential

![Measured CNR Values vs. Potential](image)
5. Effect of Nanoparticle shape and size on image contrast

Figure 5.11: Measured contrast-to-noise ratios from images obtained at tube potentials between 40 and 100 kVp. CNR values are reported for AuNP suspensions, iodinated CM, and water.

Image results and accompanying contrast-to-noise ratios are shown in Figure 5.10 & Figure 5.11. Contrast enhancement is highly uniform between nanoparticle samples. Small variations are present, but there is no consistent trend between sets of images. 3.8 nanometre spheres display highest contrast, for example, at 40 kVp but not at the other tube potentials tested. Greatest variation between CM samples occurs at 80 kVp. In this set of images, the 5.8 nm spheres and 30nm nanorods exhibit lower CNR values than the other AuNP samples. Iohexol displays greatest contrast enhancement of the measured samples at 80 kVp. This tube potential is optimal to produce photons with energy exceeding iodine’s K-edge value.

<table>
<thead>
<tr>
<th>Sample</th>
<th>40 kVp Contrast</th>
<th>CNR</th>
<th>60 kVp Contrast</th>
<th>CNR</th>
<th>80 kVp Contrast</th>
<th>CNR</th>
<th>100 kVp Contrast</th>
<th>CNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 nm Spheres</td>
<td>214.3±4.0</td>
<td>4.61±0.53</td>
<td>102.7±1.3</td>
<td>11.68±0.86</td>
<td>83.8±1.1</td>
<td>10.95±0.52</td>
<td>77.5±1.7</td>
<td>9.57±0.74</td>
</tr>
<tr>
<td>Nanorods</td>
<td>217.0±2.5</td>
<td>4.67±0.60</td>
<td>104.9±0.4</td>
<td>11.93±1.01</td>
<td>80.6±0.8</td>
<td>10.53±0.50</td>
<td>77.1±0.6</td>
<td>9.51±0.48</td>
</tr>
<tr>
<td>Iohexol</td>
<td>209.0±0.8</td>
<td>4.49±0.56</td>
<td>105.8±1.2</td>
<td>12.03±0.95</td>
<td>86.2±1.3</td>
<td>11.26±0.55</td>
<td>77.6±1.2</td>
<td>9.57±0.43</td>
</tr>
<tr>
<td>5.8 nm Spheres</td>
<td>214.5±1.5</td>
<td>4.62±0.61</td>
<td>103.8±0.7</td>
<td>11.81±1.04</td>
<td>81.1±1.8</td>
<td>10.60±0.51</td>
<td>78.1±1.0</td>
<td>9.63±0.47</td>
</tr>
<tr>
<td>3.8 nm Spheres</td>
<td>220.4±3.5</td>
<td>4.74±0.66</td>
<td>104.4±1.4</td>
<td>11.86±0.80</td>
<td>82.6±1.5</td>
<td>10.79±0.60</td>
<td>75.4±1.7</td>
<td>9.31±0.59</td>
</tr>
<tr>
<td>Water</td>
<td>196.8±3.7</td>
<td>4.23±0.48</td>
<td>93.0±1.2</td>
<td>10.59±0.98</td>
<td>71.7±0.6</td>
<td>9.36±0.33</td>
<td>70.3±1.4</td>
<td>8.68±0.56</td>
</tr>
</tbody>
</table>

Table 5.2: Measured contrast values and associated contrast-to-noise ratios for CM samples in images shown in Figure 5.10

Datasets have been compared for significant difference in contrast enhancement by multiple-measures ANOVA test (to permit comparison of grouped data; each sample at different kVp setting). Statistical analysis indicated significant pairing between CM samples (R²=0.999728, P-value <0.0001). There was no significant variation in mean CNR value between AuNP samples (P=0.6015). Detailed summary is given in appendix 10.5.2.

We anticipate a trend of declining contrast enhancement with increasing X-ray energy (kVp). This is shown in Table 5.2 with contrast described as the difference in image gray value, intensity, between background and CM regions-of-interest. Further illustration is given...
5. Effect of Nanoparticle shape and size on image contrast

in Figure 5.13. Contrast-to-noise ratio measurements are highly susceptible to the level of exposure at the image receptor and its effect on image noise. In the process of estimating an appropriate setting of current and time at each potential, it appears that the mAs setting at 40 kVp might have been slightly low. As shown in Figure 5.12, the image noise at 40 kVp is roughly 5 times greater than the other selected tube potentials. Comparison of CNR values between samples at a single potential, however, remains valid.

![Image Noise vs. Potential](image)

Figure 5.12: Measured image noise according to tube potential from the experimental images recorded in this study. Error bars represent standard deviation in noise measurements between image sets

In comparison to iodine and water control samples, contrast-to-noise ratio comparisons reveal results as anticipated. Iohexol displays comparable contrast enhancement to AuNP CM at tube potentials between 60 and 100 kVp. At these energies, the emitted X-ray spectrum contains a large proportion of photons exceeding the K-edge of iodine at 33.2 keV, improving X-ray absorption by iodine atoms by photoelectric effect.
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Figure 5.13: Measured contrast values for experimental CM samples at the tube potentials utilised in this investigation.

5.4 Conclusions

Gold nanoparticle samples of varying size distributions were successfully synthesised and concentrated to equal density of Au atoms. Based on the recorded radiographic images of the samples in this study, we cannot conclude that there is any measurable variation in X-ray absorption related to nanoparticle size. There was no evidence of distinguishable trend for contrast enhancement between nanoparticle samples. The effect of manipulating Au-Au bonding characteristics by altering the size and the shape of gold nanoparticles may have an effect on the absorption of X-ray photons when considered over very small ranges of energy (a few electron volts), but the integrated effect over a polychromatic spectrum is negligible. This is the case regardless of selected tube potential. We advise that for design of prospective nanoparticulate contrast media in size range, particle diameter and shape be tailored to achieve optimal safety and retention profiles. Concentration of radiopaque element, regardless of its atomic bonding characteristics, is the primary determinant of attenuation for
5. Effect of Nanoparticle shape and size on image contrast

X-rays in the diagnostic energy range. Further experimentation would be necessary to synthesise nanoparticles with a wider variety of dimensions. Due to the limitations of time and resources, a greater variety of nanoparticle samples could not be included in this work. Future contrast measurements would also benefit from the use of highly-concentrated AuNP samples to improve statistical significance in image measurements; permitting the detection of very small variations in X-ray attenuation.
6. Optimisation of Au Contrast Enhancement by Monte Carlo Simulation

6.1 Introduction

Monte Carlo simulation offers a powerful tool for quantifying the behaviour of radiation in materials. In this work, we have utilised these types of simulations to reproduce the task of generating X-ray photons from an electron beam, calculating the attenuation of a beam of diagnostic X-rays as it passes through CM samples and human tissue, and quantifying the response of different image receptors to the transmitted radiation. In this fashion, it is possible to recreate the full process of radiographic image-formation in a digital realm. This reduces the cost of experimentation, and—more importantly— it offers a wide variety of analytic options that are difficult or impossible to recreate in a real-world setting.

There is little published research in the field of radiographic image simulation, and even less so concerning Monte Carlo (MC) technique. A classical technique for reconstructing a radiographic image from a digital tissue phantom and a set of radiographic exposure parameters involves a ray-tracing algorithm. This type of reconstruction calculates the linear attenuation coefficient for rays extending from each image pixel to the radiation source and integrates based on a spectrum of photon fluence for a given kVp and filtration. Such a tool has been described by Lazos et al (169). Under the assumption that exponential attenuation holds true for photon intensity over the straight line passing through tissue, the transmitted intensity of the X-ray beam at each pixel has been defined based on the incident energy spectrum and the type and thickness of tissue it traverses:

\[ I = I_0 \exp\left(-\sum_{i=1}^{n} \mu_i d_i \right) \]  \hspace{1cm} (6-1)
6. Optimisation of Au Contrast Enhancement by Monte Carlo Simulation

Where:

$I_0$ is the intensity of radiation at the source that emits to the area of the X-ray detector.

$I$ is the transmitted intensity at the detector surface. $\mu t$ is the attenuation coefficient of the $i$th region.

$d_i$ is the path length through each region traversed.

$n$ is the number of regions the X-ray beam crosses.

While this type of image can be simulated relatively quickly, it lacks the robust photon transport modelling of Monte Carlo techniques. In ray-traced simulations, the transmitted photon yield through the simulated phantom lacks any scattered, secondary photons, and represents only the primary beam component of X-rays. The effects of scatter radiation must be estimated from a secondary Monte Carlo simulation and combined with the results of the ray-tracing calculation. Such simulation software has been described by several authors (169) (170) (171). The aim of such software is optimising imaging parameters to maximise image contrast for a region-of-interest while minimising patient absorbed dose, particularly to radiosensitive organs. The simulation program described by Winslow et al. is advanced in that it uses an actual 3-dimensional CT reconstruction of a human body obtained from the visible human project (a model known as VIP-MAN) to provide geometry for the ray-tracing and Monte Carlo simulations.

The majority of applications for image simulation are to improve image quality and absorbed dose in traditional radiographic procedures. This thesis represents the first application to use simulation to optimise exposure parameters for detection of experimental contrast media. The effect of the high K-edge of gold requires modelling of the generated X-ray spectra at a variety of potentials and comparison of the beam attenuation by both soft tissue and gold atoms. The previously-developed software packages (as described above) are designed to estimate X-ray transmission through a geometry described by pre-set anatomical
models. In order to incorporate regions of high-Z elemental contrast media, it was determined to be more effective to run the simulations entirely in a Monte Carlo software package.

EGSnrc Monte Carlo software package was chosen for modelling radiographic imaging procedures. EGS (Electron Gamma Shower) has been developed by the National Research Council of Canada over the last 25 years (172). It is designed to model a number of different photon and electron interactions (high-energy pair- and triplet-production, photo-electric absorption, Compton and Rayleigh scattering) and all associated secondary particles over energies ranging between 1 keV and several hundred GeV in energy (173). The probabilities of each event for a given energy, particle- and material-type have been tabulated and may be selectively loaded into the software’s memory. When running, the Monte Carlo software simulates the path of an electron or photon until it has lost its energy or left the simulation geometry. Each particle simulated in this manner is known as a “history”, and its probability of attenuation or transmission is dictated by values given in a random number generator (RNG). Each simulation is then completed on a particle-by-particle basis for millions or billions of histories.

EGS runs on the Mortran3 code, but may be implemented using graphical user interfaces (GUIs). The Mortran language (a modified version of Fortran77) provides an efficient template for running these types of calculations. Other Monte Carlo software, such as Penelope, runs on a similar Fortran-based framework (174). EGS is commonly used for both low- and high-energy applications. It has been benchmarked for the simulation of X-ray energy spectra by directing a beam of incident accelerated electrons onto a variety of angled metallic surfaces (175-177). The EGSnrc package is composed of several different sub-components, each with different capabilities in terms of recording dose profiles, describing simulation geometry, and recording transmitted photon or electron histories. This software is
6. Optimisation of Au Contrast Enhancement by Monte Carlo Simulation

the same as used to simulate the scattered photons in the ray-tracing programs described by Lazos et al., Sandborg et al., and Winslow et al. (169-171).

There is limited literature discussing the simulation of radiographic image formation using Monte Carlo technique. In large part, this is because Monte Carlo simulations suffer from inherent inefficiency issues. MC simulations very nearly reproduce the probabilistic behaviour of actual x-rays and gamma rays for a defined quanta of photons, but this requires that each particle be simulated one-by-one. At each event –either a material boundary crossing or attenuation event– the RNG must be called to dictate the particle’s next outcome (i.e. distance before depositing energy, generation of secondary particle with an associated energy and direction, etc). With modern computer processors, simulating several thousand photons takes only a matter of seconds, but the accurate reproduction of an image takes several orders of magnitude greater number of histories and (more importantly) time. As a result, efforts to simulate radiographic procedures solely by Monte Carlo technique rarely reproduce full images. Rather they approximate the dose to larger regions of the image receptor to alleviate the statistical issues that result from the random nature of the simulation (108, 178).

In the process of this research, significant effort has been placed into designing a streamlined set of parameters to simulate image formation. This has allowed the quantification of contrast enhancement for a given CM material within a time span of approximately one day on a normal personal computer (1.86 GHz Intel ® CPU).

6.2 Materials and Methods

6.2.1 Generation of X-ray Energy Spectra

Generation of X-ray photons (the conversion of accelerated electrons into electromagnetic energy) is the most inefficient of the processes that require simulation. In a
radiograph, only a small percentage of the photons leaving the tube will interact unattenuated with the image receptor. Even that percentage is very large compared to the fraction of electrons travelling in the vacuum tube that actually result in the emission of X-ray photons. Depending on their energy, accelerated electrons impinging on the tungsten target may generate Bremsstrahlung or characteristic X-rays about 5% of the time. Of those, some will be attenuated before leaving the anode surface and only half will be directed in a downward direction (vastly fewer passing freely through the leaves of the collimator). A detailed discussion of efficiency in Monte Carlo simulation of radiographic images is given in appendix 10.3.

Figure 6.1: Diagram of X-ray tube geometry used in MC simulation. A histogram of the photons passing the scoring plane (shown at z=10 cm) and their associated energies are recorded in this phase of the simulation.

In order to simulate the emission of X-ray photons in an efficient manner, radiographic MC simulations were separated into two –and sometimes three– discrete stages.
For all Monte Carlo experiments described in this work, the X-ray tube was simulated individually. Separating the X-ray tube simulation from the subsequent phases of image formation has been shown in this work to improve efficiency 500-fold and was necessary to produce statistically-sound data over a practical period of computation time.

X-ray tube simulations were completed for a variety of tube potential settings. In each case a monoenergetic beam of electrons (with kinetic energy determined by the selected tube potential; voltage waveform has been neglected) was directed at an angled tungsten surface, geometrically similar to an actual radiographic vacuum tube (see Figure 6.1 and Figure 6.2). A horizontal beam of electrons (40, 60, 80, 100, 120, 140, 160, or 180 keV) was directed at a 2.0 mm thick tungsten surface with copper backing (angled at 20° with respect to vertical). Emitted photons passed through 1 mm beryllium and 1 mm aluminium inherent filtration. Transmitted photons were then collimated by 5.0 mm thick lead collimator leaves. Only those X-rays spanning an angle of 29.3° in the downward direction were scored. A spectrum of generated X-rays at 140 kVp is shown in Figure 6.3. For comparison, it is plotted against spectra with the same tube potential and inherent filtration corresponding to the IPEM-78 report and the SpekCalc 1.0 software (116, 179). All three spectra show close correlation between 35 and 140 keV of energy. Only the spectrum generated by the IPEM-78 generator deviates slightly, reporting a slightly lower fluence of X-rays below 35 keV.
Figure 6.2: Graphical representation of X-ray tube geometry as used to generate X-ray spectrum files
6.2.2 Imaging Geometry

To improve efficiency, the X-ray tube was treated as a point source that emitted photons according to the energy distributions as explained in 6.2.1. Photons were emitted isotropically over an angle of 13.5° in the downward direction to cover the entire front surface of the tissue phantom without simulating a large quantity of X-rays that would not interact with the phantom or IR.

A simplified tissue/contrast phantom was designed to maximise data output and efficiency in MC simulated images. The phantom consisted of 15 cm thick volume of soft tissue (ICRU 4 component; 20 cm x 20 cm, length x width). Inside the phantom, cylindrical...
volumes of iodine and gold in water were designed (0.8 cm diameter). These regions were set to a concentration of 10% weight-to-volume ($\rho_{cm} = 0.11 \text{ g/cm}^3$, $\rho_{total}=1.11 \text{ g/cm}^3$); a concentration that would sufficiently attenuate the X-ray beam to display visible contrast enhancement. A similar cylindrical volume of compact bone was described into the input geometry for comparison (diameter = 4 cm). A graphical representation of the tissue phantom and imaging geometry is given in Figure 6.4.

X-rays transmitting through the phantom were scored in a phase-space file that recorded their position, energy, and direction of travel prior to interacting with the image receptor. Scoring the photons in this manner improved efficiency in simulating similar exposure conditions (e.g. the same phantom and tube potential) with a variety of image receptors (results in 6.3.2).

Figure 6.4: Orientation of tissue phantom, contrast media, and bony feature as utilised on Monte Carlo simulated images. The incident X-ray beam (from top) is transmitted or attenuated by regions before being recorded in the scoring plane.
6. Optimisation of Au Contrast Enhancement by Monte Carlo Simulation

6.2.3 Dose Distribution at Image Receptor

Image formation was recorded by describing an image receptor (IR) composed of uniform rectangular voxels with the software dosxyz (a component in the EGSnrc package) (173). In these simulations, three different IR materials were simulated; Barium-fluorobromoiodide (BaFBrI), Caesium Iodide (CsI), and Gadolinium Oxysulphide (Gd$_2$O$_2$S). BaFBrI is the same phosphor material present in the CR cassettes from the radiographic study reported in chapter 2. Gd$_2$O$_2$S and CsI are both commonly-utilised fluorescent materials employed in scintillating X-ray detectors. Images were simulated for each IR material at thicknesses of 0.1 to 1.0 mm in 0.1 mm increments to examine any trend in changing enhancement with adjustment of IR thickness. Photons scored in the phase-space file from section 6.2.2 were directed upon the image receptor. Image gray value (pixel colour/darkness) is considered directly proportional to the dose deposited in the image receptor.

6.2.4 Adjustment of X-ray Tube Potential

Images were simulated using BaFBrI image receptor (300 µm thickness) at tube potentials between 40, 60, 80, and 100 kVp. These potentials were chosen to correspond with experimental images captured previously (see section 5.2.4 Imaging protocol). A second series of images were simulated at greater tube potentials using a 1.0 mm thick gadolinium oxysulphide image receptor as this had shown optimal detection for Au regions in preliminary datasets. 1.0 mm Gd$_2$O$_2$S images were simulated at tube potentials of 80, 100, 120, 140, 160 and 180 kVp to cover a range of X-ray energies above the Au K-edge. All images were simulated based on 100 million histories incident upon the surface of the image receptor (at 120 kVp: 1.5 billion histories from source), requiring a computation time between 10-18 CPU hours depending on tube potential.
6.2.5 Image Contrast Analysis

Dosxyz dose distributions in the image receptor were imported into Matlab software (Mathworks™, V7.4 R2007a) for image reconstruction and analysis. A detailed protocol and associated scripts are described in Appendix 10.4 “Analysing simulated images from dosxyz *.3ddose files”. Simulated images were quantified by measurements of contrast ($\Delta I$) and noise ($\sigma$) to yield contrast-to-noise ratio. Regions of interest (6 pixels x 14 pixels, h x w) were selected spanning the centre of each CM sample and the region of bone. Corresponding background ROIs were selected in the adjacent regions. Contrast ($\Delta I$) was quantified as the difference in mean pixel dose between CM and background ROIs. Noise ($\sigma$) was measured as the mean standard deviation value of the two corresponding background ROIs for each contrast material (Au, I, or bone).

6.3 Results and Discussion

6.3.1 Effect of Tube potential

Radiographic images were successfully simulated by Monte Carlo technique. Figure 6.5 shows a simulated radiograph at 140 kVp. Regions of gold, bone, and iodine can be clearly visualised (though these are highlighted in the figure). Pixel gray values are representative of the radiation dose to the corresponding volume of image receptor (given in Gy per incident photon). Dose at the pixels underneath the contrast materials is measurably reduced. Those values are quantified by selection of ROIs and reported as contrast-to-noise ratio.
Figure 6.5: Simulated radiographic image at 140 kVp recorded as dose distribution on 0.5 mm thick gadolinium oxysulphide image receptor. Each pixel represents 1mm$^2$, yielding a total image area of 100 mm$^2$. Cylindrical regions of 10% Au and I are labeled (oriented vertically in images). A region of compact bone has been shown.

Radiographic images were simulated with varying tube potential under two conditions. One set of images was produced as the dose distribution from X-ray photons onto a 0.3 mm BaFBrI (coinciding with the projection imaging conditions described in Chapter 2). Images and tabulated data are given in Table 6.1 and Figure 6.6. A second set of images were simulated using an image receptor with 1.0 mm gadolinium oxysulphide with the aim of optimising Au detection compared to bony tissue.
Images simulated on BaFBrI image receptor show close correlation to data from the phantom study reported in chapter 2. It must be recognised that the images recorded in chapter 2 compared AuNP and iodinated CM at equimolar concentration (~50% greater density of iodine to gold) and a direct comparison of CNR values between studies is not applicable. Images were simulated between 40 and 100 kVp. At 40 kV, gold produces a greater contrast-to-noise ratio than the iodinated region-of-interest. This is attributed to the 40 kV energy spectrum containing a significant proportion of photons in the optimal energy range for absorption by photo-electric effect due to the 11.9 keV Au L-edge. At 40 kVp, only a small fraction of X-rays have energy exceeding iodine’s K-edge at 33.2 keV, but at tube
6. Optimisation of Au Contrast Enhancement by Monte Carlo Simulation

potentials of 60 and 80 kVp the spectrum shifts significantly above that threshold. At 80 kVp, it is shown that iodine shows greatest enhancement relative to gold with a CNR value that is 72% greater. Again, this correlates closely with findings from projection and CT radiographs where equimolar samples of AuNPs and iopromide displayed similar contrast enhancement at 80 kVp (previously discussed in section 2.3.1), but gold samples displayed significantly higher visualisation at all other energies.

<table>
<thead>
<tr>
<th>Tube Potential</th>
<th>Au Contrast</th>
<th>Noise</th>
<th>CNR</th>
<th>I Contrast</th>
<th>Noise</th>
<th>CNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.1311</td>
<td>0.0129</td>
<td>10.16279</td>
<td>0.1067</td>
<td>0.0139</td>
<td>7.676259</td>
</tr>
<tr>
<td>60</td>
<td>0.3226</td>
<td>0.0258</td>
<td>12.50388</td>
<td>0.3666</td>
<td>0.0245</td>
<td>14.96327</td>
</tr>
<tr>
<td>80</td>
<td>0.3230</td>
<td>0.0381</td>
<td>8.47769</td>
<td>0.5155</td>
<td>0.0354</td>
<td>14.56215</td>
</tr>
<tr>
<td>100</td>
<td>0.4729</td>
<td>0.0471</td>
<td>10.04034</td>
<td>0.5821</td>
<td>0.0459</td>
<td>12.68192</td>
</tr>
</tbody>
</table>

Table 6.1: Data from EGSnrc simulation of exploratory CM materials 10% (w/v). These images captured at a variety of tube potentials with a CR photo-stimulable phosphor cassette (300 µm thick BaFBr/I image-resolving layer)

Images simulated at greater tube potentials (up to 180 kVp) are described from a beam of X-rays incident on the digital tissue phantom and recorded as dose distribution on a 1.0 mm thick Gd$_2$O$_2$S image receptor. This type of IR had shown improved Au detection in preliminary Monte Carlo simulations. A detailed series of simulations relating to image receptor material and thickness is described in section 6.3.2. In these images, contrast enhancement has been quantified for gold, iodine, and bone ROIs. CNR values are reported in Figure 6.7 and Table 6.2. For all materials, CNR values steadily decline with increasing tube potential. At equal density, gold shows improved enhancement compared to iodine at 140 kVp and above as shown in Figure 6.7. CNR values of compact bone region are highest of the materials at all tube potentials. As designed in the simulation, the bony region presented a larger diameter of material (greater thickness) for the attenuation of X-rays when compared to the CM samples (4 cm vs. 0.8 cm).
### Table 6.2: Image contrast, noise, & CNR values recorded in MC simulated images with varying potential. Data are reported for gold, iodine, and bone ROIs in images simulated on 1.0 mm gadolinium oxysulphide image receptor.

<table>
<thead>
<tr>
<th>Tube Potential (kVp)</th>
<th>Au Contrast $\Delta I$ (Gy per incident photon $*10^{-14}$)</th>
<th>Au Noise (Gy per incident photon $*10^{-14}$)</th>
<th>I Contrast (Gy per incident photon $*10^{-14}$)</th>
<th>I Noise (Gy per incident photon $*10^{-14}$)</th>
<th>Bone Contrast (Gy per incident photon $*10^{-14}$)</th>
<th>Bone Noise (Gy per incident photon $*10^{-14}$)</th>
<th>Au CNR</th>
<th>I CNR</th>
<th>Bone CNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.2039</td>
<td>0.0234</td>
<td>0.2688</td>
<td>0.0216</td>
<td>0.4221</td>
<td>0.0206</td>
<td>8.7268</td>
<td>12.4378</td>
<td>20.4835</td>
</tr>
<tr>
<td>100</td>
<td>0.2797</td>
<td>0.0337</td>
<td>0.3339</td>
<td>0.0329</td>
<td>0.5691</td>
<td>0.0321</td>
<td>8.2931</td>
<td>10.1446</td>
<td>17.7568</td>
</tr>
<tr>
<td>120</td>
<td>0.3288</td>
<td>0.0435</td>
<td>0.3694</td>
<td>0.0433</td>
<td>0.6768</td>
<td>0.0443</td>
<td>7.5533</td>
<td>8.5320</td>
<td>15.2667</td>
</tr>
<tr>
<td>140</td>
<td>0.3612</td>
<td>0.0507</td>
<td>0.3873</td>
<td>0.0554</td>
<td>0.7312</td>
<td>0.0511</td>
<td>7.1315</td>
<td>6.9887</td>
<td>14.3081</td>
</tr>
<tr>
<td>160</td>
<td>0.4021</td>
<td>0.0581</td>
<td>0.4044</td>
<td>0.0613</td>
<td>0.8244</td>
<td>0.0630</td>
<td>6.9155</td>
<td>6.5916</td>
<td>13.0927</td>
</tr>
<tr>
<td>180</td>
<td>0.4185</td>
<td>0.0709</td>
<td>0.3873</td>
<td>0.0721</td>
<td>0.8725</td>
<td>0.0668</td>
<td>5.8999</td>
<td>5.3729</td>
<td>13.0596</td>
</tr>
</tbody>
</table>

The inclusion of a region of bone permits some comparison between contrast enhancement from high-Z CM and contrast from normal anatomical features. Maximising the
CNR of a CM material (which will generally occur at a very low tube potential) is of little assistance if contrast is also enhanced in background regions making detection against confounding anatomical features more difficult. Figure 6.8 describes data from simulated radiographs in which contrast in CM regions is compared to the osseous (bone) ROI (for images taken with 1.0 mm Gd₂O₂S image receptor). Image contrast (ΔI) is compared for regions of contrast media and bone. It is shown that peak enhancement for Au relative to bone occurs at approximately 140 kVp. For the iodinated ROI, greatest enhancement relative to bone is reported at 80 kVp; the lowest potential measured in this series of images.

Again, these results are in approximate accordance with measured images in computed tomography (Chapter 2). It was shown that the HU density of gold was stable at high tube potentials (up to 140 kVp, the maximum value selectable) while iodine-based CM displayed declining enhancement when high-energy X-rays were used. The same trend in these simulated images is observed. It is evident that optimal exposure parameters for iodinated CM require tube potentials in the range of 60-80 kVp (as shown in Figure 6.6), but detection of Au requires higher-energy photons; those emitted at a potential near 140 kVp. At 140 kilovolts, a significant proportion of the X-ray photons that interact with the image receptor occur above the Au-K-edge. These photons are highly penetrating to normal tissue (materials with a comparatively low effective-Z value), but readily absorbed by gold atoms. For example, the mass attenuation coefficient of gold is 27.8 and 30.5 times greater than bone and soft tissue, respectively, when considering photons at 100 keV (10).
As X-ray energy is increased to potentials above 140 kVp, we find decreasing contrast enhancement for Au relative to bone. The K-edge effect is more substantial for photons with energy close to the K-shell binding energy of Au. At significantly higher X-ray energy, the difference between gold and other human tissues declines. At 150 keV, the mass attenuation coefficient of gold is only 12.5 times greater than bone and soft tissue and at 200 keV it declines further to a factor of approximately 7 times (10). According to the findings and results in these sections, it is suggested that the ideal means of implementing AuNP contrast media in radiography is to select a tube potential for which emitted photons are predominantly in the range of 80-100 keV with a very narrow energy distribution (quasi-monoenergetic).
6. Optimisation of Au Contrast Enhancement by Monte Carlo Simulation

6.3.2 Effect of Image Receptor

Simulated radiographs were generated by Monte Carlo technique from a beam of X-rays at 140 kVp incident upon a digital tissue phantom and recorded on a variety of image receptor types. Results are presented for images recorded on Bariumfluorobromoiodide (BaFBrI – a material commonly utilised for computed radiography storage-phosphor cassettes), Caesium Iodide (CsI), and Gadolinium Oxysulphide (Gd₂O₂S). The latter materials are often utilised in the scintillating image receptors designed for direct radiography (DR) or CT (114, 125, 180). It is speculated here that altering both material type and thickness affects the measured visualisation of Au by improving absorption of high energy X-rays (> 80.7 keV). Several simulated images are reported in Figure 6.9.
Figure 6.9: Simulated images at 140 kVp with different combinations of image receptor material and thickness.

Images produced by this study indicate that image receptor thickness does have an effect on the detection of gold material relative to bone. Results of quantified contrast in the Au ROI relative to bone are given in Figure 6.10. Regardless of IR material, increasing thickness produces a clear trend of increasing contrast enhancement in the 10% gold region-of-interest. Optimal detection of Au requires that the IR be sensitive to photons in the range of 80-100 keV (in order to detect the transmission of X-rays in non-Au regions and the differential absorption by K-edge effect in regions corresponding to gold atoms). This energy range is highly penetrating and requires a greater cross-sectional thickness of IR to be absorbed. Thin image receptors can fully capture low-energy photons, but may allow higher-energy X-rays to pass through undetected. By increasing IR thickness, one increases the detector’s sensitivity to high-energy photons (in the range of the Au K-edge), but low-energy
6. Optimisation of Au Contrast Enhancement by Monte Carlo Simulation

photons are wholly absorbed in either instance. This means that contrast from Au atoms increases by utilising a thicker IR layer (1.0 mm compared to 0.1 mm), but contrast from other structural features increases to a lesser degree. This trend is shown in Figure 6.10 where the appearance of Au regions-of-interest increases relative to bone when thicker IRs are implemented in the Monte Carlo simulations.

![Image](image.png)

**Figure 6.10:** Comparison of measured contrast in Au ROI to the value in the osseous ROI. The relative enhancement has been plotted against image receptor thickness for the IR materials studied. Greatest Au enhancement relative to bone is shown when increasing IR thickness and utilising gadolinium oxysulphide as image-resolving material.

It is found, as well, that the type of material which comprises the IR has some effect on contrast enhancement. Of the three materials selected for MC simulations, gadolinium oxysulphide displayed greatest contrast enhancement. Again, this can be anticipated when it is considered that the material is the most sensitive to photons in the 80-100 keV range above the Au K-edge. The IR records dose by absorbing incident photons, optimally by photoelectric effect. The radiopaque component of gadolinium oxysulphide is the Gd atom.
(Z=64), which has a K-edge at 50.2 keV. That is nearer the optimal range for detection of Au than the other materials which rely on iodine or a combination of barium and iodine which have lower K-edge values near 35 keV (10).

6.4 Conclusions

This work has described a novel and efficient means for simulating radiographic image formation by Monte Carlo simulation (X-ray generation, attenuation, and detection). The technique has been used to design an optimised set of exposure parameters for detection of gold nanoparticles. These experiments have shown that adjustment of tube potential and image receptor material can be utilised to improve the relative detection of Au atoms in a radiographic image. Results are in close accordance with real-world images, indicating an optimal tube potential of 140 kVp. Compared to the data reported in chapter 2, the results of this chapter are in close agreement (when the comparison of equimolar samples against equalised density is considered). Utilisation of a gadolinium oxysulphide IR was shown to detect attenuation by gold more efficiently than BaFBrI or CsI. Likewise, it is suggested that image receptors with high cross-sectional thickness (1 millimetre or greater) should be considered to efficiently absorb the highly-penetrating X-rays with energy close to the Au K-edge (in the range of 80-100 keV).
7 Gold Nanoparticles in Dual-Energy Subtraction Radiology

7.1 Introduction

Many publications point to implementation of gold nanoparticles as a directed drug delivery or contrast agent that travels to tissues of interest in small quantities. Targeted delivery will alleviate many hurdles with development of novel contrast media. It would reduce the administered dosage and related severity of dose-dependent reactions. It would permit long retention time in pathological regions while preventing bioaccumulation in healthy regions. Reduced quantities of radiopaque material also would be required, lowering the associated cost of synthesis. Moreover, it would alleviate the necessity to introduce these materials at high concentration, thereby permitting coatings to be considered primarily for biocompatibility rather than high solubility.

The issue would remain, however, of a means to detect these materials against a background of contrasting tissue features. Though X-ray images permit the evaluation of composition deep inside the human anatomy, the detection of slight amounts of contrast against the appearance of healthy anatomical features presents a considerable hurdle. The aim of this experiment is to design an optimised radiographic technique for subtraction imaging; wherein two images are captured such that the appearance of gold atoms varies highly while the appearance of normal tissue remains constant. In this case, the pixel-by-pixel subtraction of the two image sets would yield the distribution of gold exclusively.

Modern dual-energy CT imaging protocols implement simultaneous scans using two X-ray sources with different tube potentials allowing the differentiation of materials on an elemental basis. This technique has been successfully employed to highlight and digitally
remove the presence iodinated contrast media (181), and we suggest a protocol to optimise similar detection of gold. Contemporary trends in radiographic imaging are leaning toward material separation and identification in images. Dual-energy CT uses subtraction technique, or to be more specific an algorithm that compares voxel Hounsfield unit density between image sets, to identify materials based on their energy-dependent attenuation. To such ends, the equipment exploits the K-edges of elements to indicate the presence of bone, contrast media, or various other anatomical and pathological features that can be of diagnostic value to radiologists (2, 181).

We propose to utilise similar principles to those in dual source computed tomography (DSCT), specifically those of second generation DSCT equipment. These machines simultaneously record two image sets, one with high kVp and one with low kVp. In the case of some scanners, 0.4 mm of tin filtration is used on the high-energy source to improve the spectral discrepancy between image sets (2). This same technique can be applied for the detection of a heavy element such as gold, but some consideration must be made for the material’s comparatively high L-edge (11.919 keV) (9). This property produces high levels of contrast enhancement at low tube potentials which can lead to diminished visualization in a subtracted dual-energy image.

The use of filters, while being designed to minimise the appearance of regions of non-interest by removing low-energy photons, could also have the added effect of reducing patient absorbed dose. For optimisation of patient dose, it has been proposed that even with the base level of inherent and added filtration in X-ray tubes, further beam hardening reduces radiation exposure to the patient for the same intensity of radiation (or optical density) at the image receptor. For a time, erbium filters had been proposed as a method for shaping the x-ray spectrum to yield an optimal balance of image quality and patient dose (182). Use of gold and copper filters, described in this work, may have a similar effect on dose reduction.
By adding a thin gold filter in the path of the X-ray beam, a large proportion of photons exceeding the 80.7 keV Au K-edge of energy will be absorbed. Radiographic images of gold nanoparticles under these conditions are expected to have suppressed Au appearance by limiting the K-edge effect; there will no longer be differential attenuation in regions of soft tissue and CM by photons above 80.7 keV. The linear attenuation coefficients of gold and copper are shown in Figure 7.1. Both materials have similar attenuation coefficients between 12 and 80 keV (10). The main variation in the energy spectra of the Au- and Cu-filtered images will occur only for photons above 80.7 keV, where these X-rays are transmitted from the X-ray tube only in the copper image set. In this manner, the difference between the two exposures will be the inclusion of high-energy photons; exactly in the range of the Au K-edge.

![Attenuation Coefficients of Gold and Copper Filters](image)

Figure 7.1 Linear attenuation coefficients for gold and copper, the materials selected for filters in this imaging protocol.
7.2 Materials and Methods

Using EGSnrc and clinical radiographic equipment, we have investigated an imaging protocol designed to highlight gold atoms in a tissue phantom. Gold’s K- and L-edges occur at 80.7 and 11.9 keV, respectively, requiring special consideration in order to remove the element’s appearance from the low-kVp scan (10). In the investigated protocol, thin gold and copper filters are used to increase the spectral discrepancy between the low and high energy image sets. Subtraction image results show improved contrast enhancement compared to conventional imaging parameters using iodinated contrast media while still removing the appearance of confounding anatomical structures.

Filter thickness and material were determined on the basis of known attenuation coefficients and previous experimentation (97). Gold filter thickness was chosen to be 0.18 mm. This dimension removes 95.4% of incident photons at 80.7 keV. The removal of this energy range limits the appearance of gold in a radiographic image, at high tube potentials. The element copper shows similar attenuation properties to gold between the energies of 12 and 80 keV, albeit requiring 5.4 times greater material thickness (9).

7.2.1 Imaging Procedure

Radiographic images were recorded with a Siemens FD-X digital radiographic X-ray machine. Tube potential settings were varied between 80 and 150 kVp with and without added spectrum-shaping filters (0.98 mm Cu, 0.18 mm Au). Gold nanoparticle suspension was obtained from Nanoprobes, Inc (Yaphank, NY 11980, USA). Iodinated CM (Omnipaque™, Iohexol. GE Healthcare Pty Ltd, Rydalmere NSW 2116, Australia) was utilised for comparison. AuNP CM samples were diluted to concentrations of 160, 32, and
6.4 mg Au per millilitre. Iohexol was diluted to 32 mg I / mL. Samples were imaged in a Perspex (PMMA) contrast phantom (shown in Figure 7.2).

![Figure 7.2: Schematic of Perspex (PMMA) contrast phantom commissioned for this study. Contrast media is enclosed in a 3 mm diameter cylindrical volume at the centre of the phantom's dimensions.](image)
Figure 7.3: Contrast phantom supported by PMMA blocks. This structure falls into the pleural cavity in the anthropomorphic chest phantom shown in Figure 7.4.

Contrast media phantom was positioned on PMMA blocks (Figure 7.3). An anthropomorphic chest phantom was then placed over the CM apparatus such that the contrast sample was approximately aligned with the position of the aortic arch in the phantom’s thoracic cavity. The arrangement of phantoms, X-ray tube, and detector is shown in Figure 7.4.
7. Gold Nanoparticles in Dual-Energy Subtraction Radiology

7.2.2 Digital Image Subtraction

Direct radiography (DR) images were collected in a manner that allowed recombination of various tube potentials and the use of filter material or unfiltered beam for different contrast media and concentrations. Digital subtraction was completed accordingly: The high-kV image was inverted for colour (negative). The low kV image was overlayed on this inverted image and given a degree of transparency that equalised contrast in ossified structures of the pleural cavity (to reduce or remove the appearance of ribs that overlayed the contrast phantom). The transparency/opacity setting for this step varied between 45 and 55% depending on the image set. In this case, varying opacity is analogous to adjusting the windowing (contrast) in one of the component images. Note: varying opacity of the low-kV image allowed for the digital removal of different structures due to varying degrees of beam hardening, image receptor response to dose, and material attenuation based on photon energy.

Figure 7.4: Anthropomorphic phantom as imaged experimentally. Experimental CM is located in smaller, contrast phantom inside (hidden from view in this photo)
between images. Once satisfactory alignment and subtraction were achieved, window and level settings of the subtraction image were adjusted to enhance contrast and normalised between image sets.

Subtraction images were analysed for contrast-to-noise ratio. Seven rectangular regions-of-interest were chosen (dimensions 11 by 147 pixels) to match the size of the central region of the contrast-bearing cylinder of the phantom (ignoring the edges which presented smaller thicknesses to attenuate the X-ray beam). The sets of ROIs used for image analysis are shown in Figure 7.5. One ROI was selected over with the experimental CM sample and measured for mean gray value. Six background ROIs were chosen in the adjacent area and analysed for mean gray value and noise as standard deviation in gray value. Contrast-to-noise ratio was calculated per image. CNR was equal to the ratio of the difference in gray value between the contrast agent ROI and the mean gray value of the six background ROIs divided by the mean image noise determined from the standard deviation in background ROIs:

\[
\text{CNR} = \frac{I_{CM} - \overline{I_{BG}}}{\sigma_{BG}}
\]  (7-1)

Where \(I_{CM}\) is the mean pixel gray value in the CM region-of-interest, \(\overline{I_{BG}}\) is the mean pixel gray value in the background ROIs, and \(\sigma_{BG}\) is the mean of the standard deviation values in the background ROIs.
7. Gold Nanoparticles in Dual-Energy Subtraction Radiology

7.2.3 Monte Carlo Simulation of Subtraction Images

Simulated radiographic images were recorded using Monte Carlo technique with the EGSnrc software package. Imaging geometry consisted of a 15 cm thick tissue phantom (ICRU 4-component). A cylindrical volume of contrast media (either gold or iodine in water at a concentration of 10% w/v) was located horizontally within the central region of the phantom. This concentration provided sufficient X-ray attenuation for detectable levels of
contrast in the subtracted images. At the base of the phantom, circular rings of contrasting tissue types (soft tissue, lung, cortical bone, and muscle) were included to for comparison and later subtraction. A diagram of the tissue phantom is shown in Figure 7.7.

![Experimental X-ray Energy Spectra](image)

Figure 7.6 Filtered energy spectra at tube potentials of 120 and 160 kVp. Subtracted spectrum (representing difference between low- and high-energy images) is shown as area graph

Exposures were simulated for a parallel beam of incident X-ray photons at 4 different energy settings: 80 kVp, 120 kVp, 120 kVp with 0.18 mm added Au filtration, and 160 kVp with 0.98 mm added Cu filtration. The first two exposures were recorded with iodinated contrast media, while the latter two correspond to gold-based contrast media. For the sake of efficiency, energy spectra were collected in a separate simulation of a standard X-ray tube with tungsten anode at a 20° angle (detailed procedure given in section 6.2.1). Energy spectra were found to be in close accordance to data from the IPEM-78 report (183). Filtered energy spectra at 120 kVp (0.18 mm Au added filtration) and 160 kVp (0.98 mm Cu filtration) are
reported in Figure 7.6. Spectra have been normalised to peak at an intensity value of 1.0 (neglecting characteristic X-rays). The subtraction spectrum (representing photons that cause a variation in the low- and high-kVp image set) is also reported in Figure 7.6.

Using these dimensions, generated energy spectra display excellent separation at the K-edge of gold. This can be seen in Figure 7.6. Spectra have been normalized to overlap below 80.7 keV. With the addition of copper filtration, the 160 kVp spectrum shows close similarity at low energies to the spectrum at 120 kVp with gold filtration; the primary exception being the $K_{\alpha}$ and $K_{\beta}$ characteristic peaks from the tungsten anode. The subtracted energy spectrum, which represents the energy range responsible for variations in attenuation between the high and low kVp images, has also been plotted. In the subtracted spectrum, 89.3% of the total photon count is above 80.7 keV, the K-edge of gold.
Figure 7.7 Geometry of tissue phantom used in imaging. Images scored as pixel dose values in a 0.8 mm layer of cesium-iodide.

Images were recorded as a set of dose values per pixel within a 0.8 mm thick layer of Caesium Iodide. Each pixel had dimensions of 1 mm x 1 mm and the overall image was recorded over an area of 100 cm². Each simulation was run for 100 million photon histories incident upon the surface of the image receptor (between 1 and 2 billion histories from the source, depending on tube potential). Image sets for either CM material (gold or iodine) were normalised for contrast of human tissue “rings” and subtracted. Subtracted images were evaluated for contrast-to-noise ratio over rectangular regions of interest with dimensions of 11 x 100 pixels. Two adjacent regions on either side were selected as background and analysed for mean pixel dose and image noise (standard deviation). A third region was selected within the margins of the contrast media sample. Contrast was determined as the difference in mean pixel dose between the central region and the surrounding background ROIs.

7.3 Results and Discussion

7.3.1 Subtraction Radiographs

Images acquired on clinical DR radiographic equipment yielded high-quality subtraction images. All subtraction images were selected for optimal removal of the appearance of ribs (particularly the 4th and 5th ribs that overlay the area of exploratory contrast media). Figure 7.8 shows the result of subtracting an image at 141 kVp (Cu filtration) and 117 kVp (Au filtration) with a region of gold nanoparticles concentrated at 160 mg Au per mL (13.8% w/v). This image displayed a relatively high CM CNR with a value of 1.0956. The greatest CNR of all datasets was recorded with both subtraction images at 117
kVp with Au/Cu filtration, yielding a CNR of 1.1861. Data from subtracted image sets are given in Table 7.1.

Figure 7.8: Subtraction image of gold nanoparticles at 160 mg Au/mL obtained by exposures at 141 kVp, 0.98 mm Cu filtration and 117 kVp, 0.18 mm Au filtration. This image displayed highest CNR value of all subtraction image sets. 3 mm wide region of AuNPs can be clearly visualised with nearly complete removal of boney structures around the chest cavity.

AuNP contrast media could be identified visually at concentrations as low as 6.4 mg/mL. An image captured using the novel subtraction technique (150 kVp – Cu filtration, 120 kVp – Au filtration) is displayed in Figure 7.9. The CM sample can be faintly identified
in the centre of the contrast phantom (indicated by arrow). This indicates the feasibility of identifying AuNP contrast media at low concentration by image subtraction.

Figure 7.9: Subtraction radiograph using filtered-beam protocol for detection of AuNP contrast media. Low-concentration Au CM is shown (6.4 mg Au per mL). CM sample can be faintly visualised after digital subtraction of the ribs.

<table>
<thead>
<tr>
<th>Contrast Medium</th>
<th>High-Energy Tube Potential (kVp)</th>
<th>Added Filtration</th>
<th>Low-Energy Tube Potential (kVp)</th>
<th>Added Filtration</th>
<th>Contrast-to-Noise Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold 160 mg/mL</td>
<td>150</td>
<td>0.98 mm Cu</td>
<td>150</td>
<td>0.18 mm Au</td>
<td>1.0259</td>
</tr>
<tr>
<td>Gold 160 mg/mL</td>
<td>150</td>
<td>0.98 mm Cu</td>
<td>141</td>
<td>0.18 mm Au</td>
<td>0.9827</td>
</tr>
<tr>
<td>Gold 160 mg/mL</td>
<td>150</td>
<td>0.98 mm Cu</td>
<td>133</td>
<td>0.18 mm Au</td>
<td>0.9248</td>
</tr>
<tr>
<td>Gold 160 mg/mL</td>
<td>150</td>
<td>0.98 mm Cu</td>
<td>117</td>
<td>0.18 mm Au</td>
<td>1.0885</td>
</tr>
<tr>
<td>Gold 160 mg/mL</td>
<td>141</td>
<td>0.98 mm Cu</td>
<td>117</td>
<td>0.18 mm Au</td>
<td>1.0956</td>
</tr>
<tr>
<td>Gold 160 mg/mL</td>
<td>129</td>
<td>0.98 mm Cu</td>
<td>117</td>
<td>0.18 mm Au</td>
<td>0.9996</td>
</tr>
<tr>
<td>Gold 160 mg/mL</td>
<td>117</td>
<td>0.98 mm Cu</td>
<td>117</td>
<td>0.18 mm Au</td>
<td>1.1861</td>
</tr>
<tr>
<td>Gold 32 mg/mL</td>
<td>150</td>
<td>0.98 mm Cu</td>
<td>121</td>
<td>0.18 mm Au</td>
<td>0.5057</td>
</tr>
<tr>
<td>Gold 6.4 mg/mL</td>
<td>150</td>
<td>0.98 mm Cu</td>
<td>121</td>
<td>0.18 mm Au</td>
<td>0.0778</td>
</tr>
<tr>
<td>Iodine 32 mg/mL</td>
<td>150</td>
<td>0.98 mm Cu</td>
<td>121</td>
<td>0.18 mm Au</td>
<td>0.2065</td>
</tr>
<tr>
<td>Iodine 32 mg/mL</td>
<td>121</td>
<td>none</td>
<td>81</td>
<td>none</td>
<td>0.3748</td>
</tr>
</tbody>
</table>

Table 7.1: Contrast-to-noise ratios from subtracted radiographic images with combinations of tube potential and added filtration. Results are reported for Gold and Iodine CM samples.
Gold samples were radiographed at concentrations ranging from 6.4 to 160 mg Au per millilitre.

In comparison to iodinated contrast media, the subtraction imaging technique described provides greater contrast enhancement with use of gold nanoparticle CM. Using a protocol of 150/121 kVp with added filtration, the gold nanoparticle sample displays a 2.5 times greater CNR value than iodinated CM for samples at a concentration of 32 mg radiopaque element per millilitre. It should be considered that the Au/Cu filter imaging technique does not ideally shape the X-ray energy spectra for absorption of the iodine K-edge at 33.2 keV. A conventional subtraction procedure for identification of iodinated CM using 121/81 kVp with no added filtration yields a CNR of 0.3748. That figure is still 26% lower than the value attained by AuNPs of equal concentration using the novel subtraction technique outlined in this chapter. Figure 7.10 shows subtraction radiographs of gold nanoparticle and iohexol CM samples at 32 mg/mL. The AuNP sample can be easily identified. It is also noteworthy that there appears to be reduced contrast from anatomical features in the subtraction image recorded with Cu/Au filtration, particularly in the regions near the spinal column and the margins of the pulmonary cavity. We attribute that to the removal of low-energy photons in the images acquired with added filtration which suppresses the appearance of bony calcium-bearing structures.
Figure 7.10: Subtracted radiographs of iodinated and Au CM samples concentrated at 32 mg radiopaque element per mL. A conventional subtraction protocol is used for iodinated CM while the added filtration technique designed in this project is utilised for the AuNP image set. The region containing Au colloid can be faintly visualised (CNR = 0.51), while the iodinated sample is difficult to resolve by eye (CNR = 0.37).
CNR values are considerably lower than projection or CT images reported in chapter 2. This is to be anticipated with subtraction images. The subtraction will inherently reduce the signal in the region of contrast media. The combination of two separate images, each with some degree of quantum mottle, results in a subtraction image with greater amplitude of image noise. These factors produce a relatively low ratio of contrast divided by image noise when compared to traditional imaging techniques, however, CM samples are clearly visualised and results are in accordance with expected values.

### 7.3.2 Data from simulated images

Simulated radiographic images were successfully produced in EGSnrc. Figure 7.11 shows a MC simulated image at 80 kVp where regions of Au contrast media and rings of tissue from the digitally-constructed phantom are clearly visualised. As expected, radiation dose is lowest to pixels underneath bony tissue and contrast media. Dose is highest in the ring corresponding to low-density lung tissue. Subtraction image sets are shown in Figure 7.12 for CM samples of 10% Au and I. Iodine images are simulated at 80 and 120 kVp with no added filtration. Gold images are simulated with and without added filtration (using the Au/Cu protocol designed in this chapter). An idealised scenario with monoenergetic beams (80.5 and 80.9 keV) of X-rays is also reported.
Figure 7.11: Simulated Radiograph at 80 kVp showing region of 10% Au CM and rings of contrasting tissue types.
Figure 7.12: Simulated X-ray images and corresponding subtraction radiographs. Original images are shown in greyscale, subtraction images with false colour. A-C a traditional subtraction imaging protocol at 80 and 120 kVp with gold contrast media. D-F the same set of exposure factors at 80 and 120 kVp with iodinated contrast media. G-I Gold contrast media...
using the added filtration subtraction technique proposed by the author (120 kVp 0.18 mm Au filtration, 160 kV 0.98 mm Cu filtration). J-L Gold contrast media imaged with monoenergetic beams at 80.5 and 80.9 keV and the corresponding subtracted image.

Data from simulated images are in accordance with measurements from radiographic images (section 7.3.1) and estimates based on tabulation of X-ray energy spectra. Contrast-to-noise ratios are reported in Figure 7.13 and Table 7.2. In subtracted images, we find that the greatest CNR is achieved using mono-energetic X-rays with energy slightly above and below the Au K-edge. These images also yield near-perfect subtraction of rings from regions of lung and bone in simulated images. The subtraction images acquired using energy spectra at 120 kVp with 0.18 mm Au and 160 kVp with 0.98 mm Cu added filtration displayed second greatest contrast-to-noise ratio of the simulated datasets. The CNR value was only 38% lower than the value obtained with mono-energetic X-rays. It was also 94% and 103% greater than the values measured with gold and iodine, respectively, using 80/120 kVp with no added filtration.

Moreover, it is visually apparent that images recorded at 120/160 kVp with added filtration yielded more-complete subtraction of rings corresponding to contrasting tissues. This is particularly evident in the region of contrast media that overlays the portion of bone in the simulated radiographs. We attribute this to the utilisation of predominantly high-energy X-rays (with energy far above the K-edge of calcium) in the images with added filtration.
### Figure 7.13: Contrast-to-Noise Ratios for simulated dual-energy subtraction images using a variety of tube potentials and filtration materials

<table>
<thead>
<tr>
<th>Contrast Medium</th>
<th>Low-energy Tube Potential</th>
<th>Low-energy Added Filtration</th>
<th>High-energy Tube Potential</th>
<th>High-energy Added Filtration</th>
<th>Contrast-to-Noise ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold</td>
<td>80.5 keV (monoenergetic)</td>
<td>N/A</td>
<td>80.9 keV (monoenergetic)</td>
<td>N/A</td>
<td>1.8926</td>
</tr>
<tr>
<td>Gold</td>
<td>120 kVp</td>
<td>0.18 mm Au</td>
<td>160 kVp</td>
<td>0.98 mm Cu</td>
<td>1.1772</td>
</tr>
<tr>
<td>Gold</td>
<td>80 kVp</td>
<td>None</td>
<td>120 kVp</td>
<td>None</td>
<td>0.6078</td>
</tr>
<tr>
<td>Iodine</td>
<td>80 kVp</td>
<td>None</td>
<td>120 kVp</td>
<td>None</td>
<td>0.5803</td>
</tr>
</tbody>
</table>
Table 7.2: Contrast-to-Noise ratios for subtraction images simulated with BEAMnrc and dosxyz. Various combinations of tube potential and filter material have been incorporated to optimise the energy spectra in low- and high-energy image sets.

7.4 Conclusions

This experiment was aimed at determining a protocol for maximizing visualization of a gold-based contrast medium using standard radiographic equipment. An ideal beam of coherent, mono-energetic X-rays could be supplied by a synchrotron. Due to cost, size, and limited availability, however, such a technique is not practical for widespread clinical use. In such an idealized scenario, two scans could be performed at 80.6 and 80.7 keV, where there would be a significant change in the attenuation of gold atoms with only a marginal shift in the appearance of any other anatomical structures. A direct subtraction of those images would identify the presence of gold almost exclusively. This experiment has aimed to provide the nearest possible replication of that scenario with a standard x-ray tube.

This work presents unique approach toward radiographic detection of gold nanoparticles. Special consideration has been given to the energy-dependent attenuation of gold as an element. Relative to normal tissue, iodine has a greater probability of attenuating high-energy X-rays. Gold, however, has been shown to display similar contrast enhancement in both low- and high-energy image sets due to its 11.9 keV L-edge (results given in section 2.3.1). These radiographic images and Monte Carlo simulations indicate that it is possible to dramatically suppress the appearance of gold in a low-energy image through the use of a thin filter manufactured out of gold. At 120 kVp, the energy spectrum is shifted heavily into the range of 40 to 80 keV. Transmission of higher-energy photons is abruptly cut off by photoelectric absorption in the Au filter. By filtering the high-kV image with copper,
confounding contrast from other anatomical structures is reduced, thus improving the appearance of gold in the normalized and subtracted image set.

This study presents a new technique for measuring the presence of gold against other anatomical features in radiography. We have compared our protocol using filtered X-ray beams to a conventional method for detecting iodine. Results show superior enhancement with good removal of undesirable structures. The region containing gold nanoparticles could be clearly visualised in a 3 mm cylinder at a concentration as low as 6.4% w/v. With continued research in the design and fabrication gold nanoparticles (particularly those with cell- or cancer-specificity), a non-invasive technique for the detection of trace amounts of gold within the body will be increasingly important. Although these simulations are recorded using projection-type geometry, the principles of image-formation are analogous to those in computed tomography. A similar algorithm to those in place for separation of iodinated contrast media in CT could be determined through measurement and calibration (181). The radiographic technique proposed is both simple and cost-effective. Current dual-source CT scanners could support such an examination with only minor modification.

In future experimentation, it would be advisable to measure the emission of characteristic radiation by the added filtration material. This experiment did not show any effect on image quality by these photons, but the radiation may result in increased patient dose. The addition of a second layer of Aluminium to further filter the characteristic X-rays may be advisable. It is also notable that, though the thicknesses of filters chosen provide good spectral separation between images, they also attenuate the intensity of the incident beam significantly. At 160 kVp with Cu filtration, the beam fluence decreases by nearly 50%, while the Au-filtered 120 kVp exposure drops to just 9.6% of its initial intensity. Longer exposure times would be required to compensate without increasing image noise.
8 Monte Carlo Investigation of Gold Contrast Media in Subtraction Imaging with Double-layer Scintillator

8.1 Introduction

With the aim of identifying materials based on elemental composition and the related attenuation characteristics, there are two energy-subtraction techniques that might be employed. In the previous chapter, a dual-energy subtraction technique involving multiple X-ray exposures was discussed. In that protocol, two separate exposures were composted to record the effect of certain materials absorbing selectively more high- or low-energy X-rays. In some ways, it would be preferential to use a single exposure to capture information regarding the composition of elements based on the amplitude of absorption just above and below the K-edge values of certain materials.

Ideally, a detector would be able to resolve many different energy levels. For example, a 2-dimensional grid of CdTe detectors (similar to those used to measure X-ray spectra in chapter 3) could record an image along with the spectral distribution of transmitted X-rays at each pixel area. A functional detector of this nature has been designed, but with many shortcomings in terms of practicality in diagnostic radiology (184). Such a technique would yield considerably more information regarding elemental composition of anatomical structures in a radiograph. It could resolve absorption at the K-edge peaks of virtually any element. Quantifying the calcium content of boney tissue, for example, could be easily accomplished by analysing how much of the beam is attenuated at energies slightly above and below the element’s K-edge in a single polychromatic exposure. Unfortunately these types of detectors are limited by their maximum radiation dose rate. It would require exposures to be captured with very low tube currents over long time periods, in turn being
susceptible to motion artefacts. Our experience has shown that these detectors also have the tendency to accumulate electrical charge and lose resolution when used consistently over a period of hours, thus would not be practical for routine clinical use.

Instead it may be feasible to use an image receptor composed of multiple layers that have a bias for detecting certain ranges of X-ray energies. This is similar, in principle, to the technique used to record colour images in photography. In that case, three discrete images are recorded on separate emulsion layers of the photographic film, each sensitive to a different colour in the visible light spectrum through the use of specialised photosensitive chemicals and dyes.

A similar principle can be applied to radiographic imaging. In the case of an X-ray image receptor, low-energy photons are attenuated in the uppermost thickness of IR material (photons below 40 keV in energy are unlikely to penetrate more than 100-200 µm of most image receptor materials), while more penetrating (high-energy) photons require a greater thickness to be fully absorbed. From previous findings, it was apparent that a very thin image receptor recorded poor contrast enhancement of gold. This was attributed to the image being formed primarily by low-energy X-rays, below the element’s K-edge. As the X-rays penetrate deeper into an image receptor, these low energy photons are almost completely removed and any energy deposited in the detector will be the result of high-energy X-rays, ideally those in the range of the Au K-edge (>80 keV). By separating the image receptor into two discrete layers, one can effectively record a low- and high-energy image simultaneously with a single exposure.

Such a concept was first reported in 1995 by Kamimura and Takashima for use with photostimulable phosphor image plates (185). In their study, two PSP plates were separated by a 1 mm thick layer of copper. A single exposure then recorded images onto both plates, with the first layer representing primarily low-energy X-ray photons. The addition of 1 mm
Cu between the plates shifted the energy range of photons in the second, deeper plate. Effectively, this technique offered the benefits of dual-energy imaging (differentiation of materials based on their energy-dependent attenuation) without the problem of image registration due to patient movement between different exposures. The research indicated improved detection of pulmonary nodules compared to diagnosis with a single exposure.

Scintillation-type detectors present a potential arrangement for designing an X-ray image receptor composed of multiple layers, but certain design features should be considered. Both layers must sufficiently separate so as to prevent light or electrical charge from passing between the two. The electronics (wiring and photodiodes) must be sufficiently radiolucent to permit X-rays to pass freely into the second layer. A description of such an imaging system is given in two recent Philips patents (Koninklijke Philips Electronics N. V.) (186, 187) and in published experiments by Grinyov et al. (188, 189).

The designs reported in the Philips patents involve two layers of scintillating crystal incorporated with ultra-thin photodiodes. To improve the spectral discrepancy between the layers (i.e. such that the upper layer interacts predominantly with low-energy X-rays), the inventors suggest the used of Yttrium Aluminium Garnet ("YAG") as the component for the first detecting layer. This material has the advantage of being low in density ($\rho_{\text{YAG}} = 4.55 \text{ g/cm}^3$ (190)) and containing elements with relatively low K-edge values (Yttrium: 17.04 keV). The fractional absorption for X-rays versus energy of a variety of YAG thicknesses is shown in Figure 8.2.

The lower scintillation layer, designed to be sensitive to harder X-rays, should contain heavier elements and be of relatively high density. Altman et al. suggest the use of either gadolinium oxysulphide (Gd$_2$O$_2$S, "GOS") or cadmium tungstate (CdWO$_4$) for this purpose (186). Fractional absorption values of GOS are shown in Figure 8.3. It is clear that its higher
density and the presence of heavy elements permits this material to attenuate harder X-rays necessary to record the high-energy component of a dual-energy image.

8.2 Materials and Methods

8.2.1 Monte Carlo Simulation

Simulated radiographic images were recorded using Monte Carlo technique with the EGSnrc software package. Imaging geometry consisted of a 15 cm thick tissue phantom (ICRU 4-component). A cylindrical volume of contrast media (either gold or iodine in water at a concentration of 10% w/v) was located horizontally within the central region of the phantom. At the base, circular rings of contrasting tissue types (soft tissue, lung, cortical bone, and muscle) were included to for comparison and later subtraction. This is the same geometrical phantom described in Chapter 7. Each simulation was completed for 1 billion histories incident upon the image receptor surface.
8. Monte Carlo Investigation of Gold Contrast Media in Subtraction Imaging with Double-layer Scintillator

Figure 8.1: Rendering of MC simulation geometry used in this study. The X-ray beam is attenuated by regions of contrast media and tissue before being captured onto the layers of image receptor. For illustration purposes, scintillator planes have been separated. In simulation there is no gap between the base of the tissue phantom and the IR or its layers.

Tube potential was selected at 140 kVp. Results from Chapter 6 indicated that this potential offers a significant portion of photons exceeding 80.7 keV of energy while still being in the energy range of most clinical radiography equipment. To improve efficiency, simulation of the X-ray tube (electrons incident upon the surface of a tungsten anode) was completed separately according to the procedure described in section 6.2.1. X-rays were considered as emitted in a uniform parallel beam over the area spanning the phantom’s surface.

8.2.2 Scintillator Layer Thickness

Though the basic description of the detector in this system has been outlined by Altman et al., they do not discuss the optimal thickness of either scintillation layer (186).
This is critical as the thickness of the first layer is the primary determinant of the balance between the quantity of hard and soft X-rays that interact with either scintillator component. Moreover, the description as it appears in the patent is aimed at the separation of materials such as calcium and iodine. For detection of Au, the balance should be tailored to the element’s 80.7 keV K-edge. In this way the upper layer absorbs primarily photons with less than 80.7 keV of energy, leaving those with higher energy to be absorbed in the next section of detector.

X-ray image simulations were completed while varying the thickness of the upper YAG scintillator layer. This dimension is considered to be the determining component that dictates the balance of hard and soft X-rays absorbed by the two layers. Images were simulated for YAG layers ranging from 0.05 to 1.0 millimetres. The lower layer for all simulations was set constant at 1.8 mm GOS, a thickness shown to absorb nearly 100% of incident X-rays below 100 keV in energy (Figure 8.3).
8. Monte Carlo Investigation of Gold Contrast Media in Subtraction Imaging with Double-layer Scintillator

Figure 8.3: Calculated fractional absorption for layers of gadolinium oxysulphide (GOS) image receptor. Curves are reported for IR thicknesses of 0.05 to 5 millimetres.

8.2.3 Image Analysis

Image receptor dose distributions were imported into Matlab (Mathworks™, V7.4 R2007a) and arranged into matrices using the technique described in Appendix 10.4 (modified to accommodate the multiple IR layers). Subtraction image sets were calculated as the difference in the pixel dose values in the upper IR layer minus the associated pixel dose in the lower layer. Pixel dose in the lower layer was multiplied by a scalar of 4.5 to normalise images for even subtraction.

Subtraction images were quantified for image contrast, noise, and contrast-to-noise ratio. Regions-of-interest (100 x 11 pixels, h x w) were selected for regions corresponding to contrast media or background (18 pixels left or right of CM). Image noise calculated as the mean of standard deviation values in the background ROIs. Image contrast determined as the mean difference in pixel dose between CM and BG regions-of-interest.
8. Monte Carlo Investigation of Gold Contrast Media in Subtraction Imaging with Double-layer Scintillator

8.3 Results and Discussion

8.3.1 Subtracted Radiographs

Simulations by Monte Carlo technique with EGSnrc produced sets of projection radiographic images for a double-layer image receptor recorded with a single exposure. A subtraction image set for 10% Au contrast media is shown in Figure 8.4. Image on left shows distribution of pixels doses distributed on 0.6 mm yttrium aluminium garnet (upper) layer of the image detector. Second layer image (recorded on 1.8 mm gadolinium oxysulphide) is shown in the centre. Normalised and subtracted image is shown on the right. In both unsubtracted images the rings of bone and lung can be clearly differentiated. The appearance of Au is significantly suppressed in the upper layer compared to the lower layer. Subtraction of the first and second layer images highlights the region containing gold atoms. It was not possible to achieve complete subtraction of the circular tissue features. Regions of bone and lung appear red in the subtraction image; however the margins of these areas are poorly defined compared to the contrast media.
Figure 8.4: Simulated radiographic images produced with a double-layer image detector comprised of 0.6 mm yttrium aluminium garnet (YAG) and 1.8 mm gadolinium oxysulphide (GOS).

Analysing the subtraction image sets for contrast-to-noise ratio, we conclude that the optimal thickness of the upper (YAG) layer is approximately 0.4 millimetres. This is shown to produce a good balance of image contrast in the CM region-of-interest while minimising noise in the subtracted image set. Comparing the measured CNR values with varying YAG layer thickness, Figure 8.5 shows that contrast-to-noise ratio increases as the upper layer thickness is increased from 0.05 to 0.4 mm. As the dimensions of the YAG are increased further, CNR values appear to plateau, declining only slightly.
Figure 8.5: Contrast-to-noise ratios of gold regions of interest (10% w/v) measured in simulated radiographs. CNR values are reported for varying thicknesses of Yttrium Aluminium Garnet upper scintillation layer. Optimal thickness is shown at approximately 0.4 millimeters.

Figure 8.6: Image contrast in Au CM ROI from subtraction images simulated with dual-layer YAG/GOS image receptor. Variation in contrast is reported with adjustment of YAG (top) layer thickness from 0.05 to 1.0 millimetres. GOS (bottom) layer was constant at 1.8 mm.
Further analysis shows that peak image contrast is achieved with very thin (0.1 mm) yttrium aluminium garnet upper IR layer. Image contrast values versus cross-sectional thickness of the upper YAG layer are reported in Figure 8.6. This thickness of YAG optimally balances the distribution of soft and hard X-rays that interact with the two detector components to achieve maximum contrast due to the 80.7 keV Au K-edge. It is shown in Figure 8.7 that subtraction images simulated with very thin YAG layers are prone to higher levels of image noise. That can be attributed to the layer absorbing a small quantity of incident X-ray photons, yielding an image with greater variance in pixel dose due to quantum mottle.

A summary of the simulated radiographic image data are given in Table 8.1. Again, we suggest that the optimal dimensions of a dual-layer image detector for identification of Au in a radiograph include an upper 0.4 mm YAG layer and lower 1.8 mm GOS layer. At these thicknesses, the dual-layer image set contains an optimal balance of contrast in the Au ROI and lacks excessive image noise that can hinder identification of contrast media.
8. Monte Carlo Investigation of Gold Contrast Media in Subtraction Imaging with Double-layer Scintillator

Figure 8.7: Image noise measured from background ROIs in subtracted radiographs simulated with dual-layer IR. Results are reported for images with varied YAG layer thickness (0.05 to 1.0 mm). GOS layer was constant at 1.8 mm.

<table>
<thead>
<tr>
<th>YAG Layer Thickness (mm)</th>
<th>GOS Layer Thickness (mm)</th>
<th>Contrast $\Delta I$ (Gy per incident particle $\times 10^{-14}$)</th>
<th>Noise $\sigma$ (Gy per incident particle $\times 10^{-14}$)</th>
<th>Contrast-to-Noise Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>1.8</td>
<td>0.01231</td>
<td>0.01664</td>
<td>0.7398</td>
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<td>0.1</td>
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<td>0.01303</td>
<td>0.01415</td>
<td>0.9206</td>
</tr>
<tr>
<td>0.2</td>
<td>1.8</td>
<td>0.01208</td>
<td>0.01192</td>
<td>1.0133</td>
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<tr>
<td>0.4</td>
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<td>1.1181</td>
</tr>
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</tr>
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<td>1.8</td>
<td>0.00861</td>
<td>0.00781</td>
<td>1.1035</td>
</tr>
</tbody>
</table>

Table 8.1: Data from simulated subtraction images recorded on Dual-layer image receptor.
8. Monte Carlo Investigation of Gold Contrast Media in Subtraction Imaging with Double-layer Scintillator

8.3.2 Comparison to Iodinated CM

Double-layer detector radiographs were simulated with both gold and iodine CM regions. Images with a combination of 0.4 mm YAG and 1.8 mm GOS are shown in Figure 8.8. Images acquired for an iodine sample are given on the top row while Au CM images are shown bottom. Unsubtracted radiographs appear similar for both CM samples. Subtraction images reveal slightly brighter contrast resulting from the Au image set.

Simulated Dual-Layer Subtraction images at 140 kVp

![Simulated dual-layer subtraction images](image)

Figure 8.8: Simulated dual-layer subtraction images with experimental contrast media (Au & I, 10% w/v). Top layer component is recorded on 0.4 mm YAG (left). Images recorded on bottom 1.8 mm thick GOS are shown (centre). Subtracted image sets appear in colour on right.
Figure 8.9: Contrast-to-Noise ratios for simulated subtraction images recorded on dual-layer IR at 140 kVp. Results from comparable simulations with 0.2 and 0.4 YAG (upper) layer are given. Bottom layer is constant at 1.8 mm GOS.

Comparison of contrast-to-noise ratios for simulated images indicates that Au is the preferred radiopaque element for subtraction imaging of this nature. In both simulated imaging conditions (0.2 mm & 0.4 mm YAG, upper layer thickness) the region containing 10% gold displayed higher CNR values than a comparable region of iodine. Au, at equal density, reported a 38% greater contrast-to-noise ratio than iodine using a scintillator composed of 0.4 mm YAG and 1.8 mm GOS (the optimal dimensions as reported in 8.3.1). A summary of image contrast, noise, and CNR data are given in Table 8.2.

We propose that gold is an optimal element for use as contrast medium in this form of single-exposure dual-energy subtraction radiography. Compared to iodine, Au has a significantly higher K-edge value than conventional iodinated compounds (80.7 keV vs. 33.2 keV). With a K-edge that lies at a high energy in the diagnostic range, the appearance of Au
can be more easily separated in a low- and high-energy image set. Because the K-edge of iodine is just 33.2 keV, the low-energy image component for detecting iodine should be produced predominantly by photons with less than 33 keV of energy. Considering an exposure in the range of 100-140 kVp, only a small percentage of these soft X-rays are likely to penetrate a patient and subsequently contribute to image-formation.

<table>
<thead>
<tr>
<th>YAG Layer Thickness (mm)</th>
<th>GOS Layer Thickness (mm)</th>
<th>Contrast Medium</th>
<th>Contrast ΔI (Gy per incident particle *10^-14)</th>
<th>Noise σ (Gy per incident particle *10^-14)</th>
<th>Contrast-to-Noise Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1.8</td>
<td>Au 10%</td>
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<td>1.0133</td>
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<tr>
<td>0.4</td>
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<td>Au 10%</td>
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<tr>
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<tr>
<td>0.4</td>
<td>1.8</td>
<td>I 10%</td>
<td>0.00818</td>
<td>0.01010</td>
<td>0.8099</td>
</tr>
</tbody>
</table>

Table 8.2: Data recorded from simulated dual-layer images with gold and iodine CM.

### 8.4 Conclusions

Simulated radiographs have shown that subtraction images can be captured with a single exposure using a multi-layered detector array. Images indicate that gold could be easily visualised at a concentration of 10% w/v. Regions of gold contrast media displayed greater contrast enhancement than comparable regions of iodine. Due to the very high K-edge of Au, we propose that it is an optimal element for consideration as a contrast medium in this form of radiography. Though the manufacturing techniques for production of a scintillator of this nature have only been recently described (186, 187), the technique would greatly improve information acquisition in radiographic exposures.

These experiments have expanded upon the basic schematics described by Altman and Levene (186, 187) to indicate the optimal detector dimensions to maximise spectral separation between images to highlight the K-edge of Au. We propose an upper (first layer) comprised of low-density yttrium aluminium garnet with a thickness of 0.4 millimetres in
order to capture an appropriate range of soft X-rays while mitigating image-noise. The second layer would ideally be manufactured from gadolinium oxysulphide, a material with high X-ray absorption coefficients in the range above the Au K-edge. It is suggested that this second layer be in the range of 1.8 mm in thickness. Though these experiments have been designed for the implementation of Au contrast media, a similar protocol could be applied to the detection of other types of radiopaque CM (iodine, gadolinium).
9 Conclusions

9.1 Summary

This work has yielded several worthwhile features regarding the implementation of gold nanoparticles in radiographic imaging. At the time this project had commenced, there had been one publication relating to the use of gold nanoparticle CM in radiology (64). Results from that study were considered only for a mouse model and using radiographic exposure factors that would be inappropriate for clinical contrast-aided procedures (22 kVp). Though the K-edge of gold and associated attenuation coefficients have been long documented (191), previous literature regarding the use of gold nanoparticles in X-ray imaging had made no consideration for the optimal exposure conditions to maximise contrast enhancement for these novel materials. The experiments reported in Chapter 2 have quantified contrast enhancement by AuNPs at a variety of tube potentials with an appropriate amount of tissue-simulating scatter material to simulate the conditions of clinical procedures. We report that gold nanoparticles most-efficiently attenuate X-rays when imaged with tube potentials below 40 kVp or above 100 kVp. In these scenarios, the polychromatic energy spectrum of X-rays emitted by the tube will fall into the appropriate range of wavelengths to maximise absorption by L- or K-edge effects.

These conclusions are supported by energy spectrum measurements quantified by CdTe detector and diagnostic X-ray equipment. Data from spectral measurements and calculated attenuation by contrast medium samples (gold and iodine) has been described in chapter 3. Furthermore, simulated radiographic images produced by Monte Carlo technique identify optimal Au contrast enhancement relative to human bone at a tube potential of 140 kVp. Under these conditions, the range of X-ray energies responsible for exposing the pixels
9. Conclusions

of the image receptor lie heavily in the range of 80-100 keV; optimally above the K-edge of gold.

A protocol for simulating X-ray images by Monte Carlo technique was developed specifically for this research. Special consideration was given to separating and streamlining the inefficient phases of X-ray image formation to yield statistically-sound data in a practical time-frame. Monte Carlo simulation has indicated gadolinium oxysulphide to be an improved image-resolving material when considering image receptor for detection of attenuation by gold. Our results indicate GOS displays greater sensitivity to photons in the range of 80-100 keV than either CsI or BaFBrI. Simulated images found greatest CNR using a 1.0 mm thick layer of Gd$_2$O$_2$S to detect X-rays in the IR, though greater thicknesses (up to 2 millimetres) may be preferential to improve fractional absorption of this range of relatively hard X-ray photons.

An innovative technique for highlighting the presence of Au by single-exposure subtraction imaging is described through the use of a dual-layer detector. MC simulation has indicated an IR composed of 0.4 mm yttrium aluminium garnet and 1.8 mm gadolinium oxysulphide yields a peak contrast-to-noise ratio in a subtracted image of gold and anatomical tissue. This method of forming radiographic “spectral” images is still in investigative stages, but we have identified that gold is an optimal element for detection by such means. That is attributed to the material’s very high K-shell binding energy compared to conventional iodinated CM or the elements that represent the primary components of human tissue.

A novel method of filtered-beam dual-energy subtraction radiography was investigated both experimentally and by simulation. Findings from these measurements have shown that gold nanoparticles can be detected by dual-energy subtraction, however image quality is improved through the use of added filtration on the low- and high-energy image sets. That creates a greater variation in the energy spectra used to capture these two
component images; with emphasis on the range of energies slightly exceeding the Au K-edge. The technique also indicated improved subtraction (removal on the image) of unwanted contrasting anatomical features. From experimental and simulated data, it was shown that a technique of 120 kVp with 0.18 mm Au added filtration and 140-160 kVp with 0.98 Cu added filtration produced high-quality subtraction radiographs. The contrast-to-noise ratios of gold ROIs for this protocol were greater than a comparable technique (80/120 kVp) with iodinated CM.

Portions of this project have given consideration to nanoparticle-specific variations in X-ray absorption. These were examined by X-ray absorption spectroscopy of the Au L$_3$-edge at the Australian synchrotron. Though there was some variation in the amplitude of the linear attenuation coefficients of AuNPs and Au reference foil when considered over small ranges of energy (less than 50 eV), the overall absorption of a polychromatic spectrum of X-rays did not yield a measurable variation between very small and very large nanoparticles. XAS also permitted structural evaluation of nanoparticles following neutron irradiation. Results from this experiment did not elicit any detectable structural effects due to the neutron capture and decay of $^{197}$Au atoms to $^{198}$Hg. This was the first experiment of this nature. For any future work in the field, we advise that nanoparticle samples receive radiation from a very high-intensity source to increase the fraction of gold nuclei converted to mercury.

We conclude that gold nanoparticle suspension could be considered as a highly-effective radiopaque contrast agent. Further study into the material’s acute and long-term toxicity is required, however AuNP contrast could be a viable alternative in the cohort of patients presenting with risk factors for iodine-induced adverse events (age, renal insufficiency, previous reactions). Current trends in research also point to implementation of AuNP CM as a targeted or functional imaging agent in radiology (103). Such techniques would extend the capabilities of contrast-aided CT beyond what is currently capable with
iodinated CM. The innovations in terms of Au detection optimisation that have been outlined in this project can be utilised in any application of gold nanoparticle contrast media.

9.2 Future Work

Validation of Monte Carlo imaging experiments would be valuable. Dual-energy subtraction with gold and copper filtration has been tested using projection DR equipment, but the most valuable application of this exposure technique would be in computed tomography. These experiments could be completed on current dual source CT equipment. Such an experiment would require the modification of the machine by introducing the added filtration in the line of the beam (preferably affixed near the exit point of each X-ray tube). It would also require the operator to be able to manually adjust mAs to compensate for the decrease in beam intensity.

Throughout this research, the project was hindered by a limited availability of suitable concentrated nanoparticles. As the research progressed, we were fortunate enough to form a collaboration with two experts in the synthesis of metallic nanoparticles, but even so, the prospect of producing gram-scale quantities of stabilised, dispersible gold nanoparticles was far beyond the time and resources available in this project. There are dozens of publications describing different synthesis procedures for gold nanoparticles of various shapes and sizes with a multitude of capping agents, but very few describe the production of a hydrophilic species that forms a stable suspension at high concentrations. This is a massive hurdle and future research in the area should consider a practical means of engineering these materials.

Unfortunately beyond the scope of this project, strong efforts to synthesis non-toxic, targeted gold nanoparticles would be essential to improve the possibility of using these materials in diagnostic radiology. In practice, it seems the most viable application for
9. Conclusions

Radiopaque gold nanoparticles would employ monoclonal antibody-bound species that have high specificity for target cells (malignancies, select tissue types, etc). There would be several advantages to such a material. First, the amount of material to be administered would be vastly reduced. That would remove the requirement to introduce the CM at high concentration. It would also drastically lower the mass of gold required per procedure; keeping cost down and assuage any stigma regarding nonreturnable consumption of a precious metal (which would otherwise be in the range of grams per procedure). Pharmaceutical toxicity is often dosage-dependent (as seen with iodinated CM adverse events), so reducing the administered dosage is expected to reduce toxicity. By binding to cell membranes or being absorbed through phagocytosis, it is expected that the retention time of these conjugated-type nanoparticles would also increase.

Dedicated in vitro and in vivo toxicity analyses would be valuable to assess the clinical viability of any candidate AuNP contrast medium. Optimally a long-term live animal study would be used to measure the retention of these materials over periods of weeks or months. Likewise, tissue assays of sacrificed animals could be worthwhile to study excretion routes, agglomeration, and permeability with respect to the blood brain barrier. These are areas that can provide insights regarding the materials safety profile and can be dependent on nanoparticle size and capping moiety.

This research has shown that gold as an element can be selectively detected using X-ray imaging at levels comparable to if not better than those achieved with iodinated CM. We propose several techniques to improve detection of gold nanoparticles in radiology; potentially improving the viability of this form of CM and encouraging further research in AuNP syntheses, pathology-specificity, and toxicity.
References

References


78. Mandal SK, Nath TK, Das AK, Karmakar D. Microstructural, magnetic, and optical properties of Zn (MnCo) O (x= 0.1 and 0.2) semiconducting nanoparticles. Journal of Applied Physics 2007; 101:063913.


90. Moniz E, Pinto A, Lima A. A Roentgenpraxis 1931; 4:90.
References

136. Bus E, Bokhoven JAv, Prins R. Synthesis of gold nanoparticles on TiO2 studied with Quick EXAFS.
References


References


References

## 10 Appendices

### 10.1 $^{197}$Au Neutron Capture Rate Data

<table>
<thead>
<tr>
<th>Neutron Energy (MeV)</th>
<th>Normalised Fraction of total Flux</th>
<th>Rate of Neutron Emission at energy range (n/sec)</th>
<th>Au (n,2n) Absorption Cross Section (barns)</th>
<th>Rate of fractional absorption (# of absorption events / # of Au atoms * sec)</th>
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**Total Fractional Absorption Rate**

(# of events / Au) = 3.76072E-21
10.2 Au ATOMS/FEFF Input Data

The following input data were used to create radial distribution functions from published Au crystallographic parameters at room temperature (138). For the purpose of this experiment, the ATOMS code has been called using the ARTEMIS GUI. The structural parameters described by Suh et al. are shown in Figure 10.1 after being input into the interface. The coding from the ATOMS output file is shown below:

![Figure 10.1: Au input parameters used to describe scattering paths in ATOMS using the ARTEMIS graphical user interface.](image)

* This feff6 input file was generated by Artemis 0.8.014
* Atoms written by and copyright (c) Bruce Ravel, 1998-2001

* total mu*x=1: 2.79 microns, unit edge step: 4.76 microns
* specific gravity = 19.399
* Normalization correction: 0.00042 ang^2
The following crystallographic data were used:

* title Gold
* title Au
* title Suh, I.-K.
* title High-temperature thermal expansion of six metallic elements measured
* title by dilatation method and X-ray diffraction
* title Locality: synthetic
* title Sample: at T = 293 K
* space = F m -3 m
* a = 4.0720 b = 4.0720 c = 4.0720
* alpha = 90.0 beta = 90.0 gamma = 90.0
* core = Au edge = L3

atoms
* ! elem x y z tag occ
  * Au 0.00000 0.00000 0.00000 Au 1.00000

TITLE Gold
TITLE Au
TITLE Suh, I.-K.
TITLE High-temperature thermal expansion of six metallic elements measured
TITLE by dilatation method and X-ray diffraction
TITLE Locality: synthetic
TITLE Sample: at T = 293 K

HOLE 4 1.0 * Au L3 edge (11919.0 eV), second number is S0^2

* CONTROL mphase,mpath,mfeff,mchi
* PRINT 1 0 0 0
* RMAX 5.50

* CRITERIA curved plane
* DEBYE temp debye-temp
* NLEG 4

POTENTIALS
* ipot Z element
  0 79 Au
  1 79 Au

ATOMS * this list contains 43 atoms
* x y z ipot tag x distance
  0.00000 0.00000 0.00000 0.00000 0.00000
  2.03600 2.03600 2.03600 1 Au_1 2.87934
-2.03600 2.03600 2.03600 1 Au_1 2.87934
  2.03600 -2.03600 2.03600 1 Au_1 2.87934
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  0.00000 0.00000 4.07200 1 Au_2 4.07200
  0.00000 0.00000 -4.07200 1 Au_2 4.07200
  4.07200 2.03600 2.03600 1 Au_3 4.98716
-4.07200 2.03600 2.03600 1 Au_3 4.98716
Based on scattering events for atoms within 5.5 Å of the absorbing Au atom, 7 discrete paths were reported. Three primary paths (those with greatest amplitude near the appropriate R-space range) have been shown in bold:

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10.3 Efficiency issues in Monte Carlo simulations

The EGSnrc and BEAMnrc package includes simple component modules that allow users to accurately simulate the geometry of an x-ray tube and the subsequent distribution of photons that it produces. In the process, an incident beam of electrons is directed at an angled tungsten surface, angled at 20 degrees with respect to the vertical axis in this example. Treating the electron beam as mono-energetic (a close approximation to modern 3 phase x-ray tubes), these charged particles will interact with the tungsten atoms on the surface of the simulated anode (shown as yellow in the diagram, the copper support shown as orange). Bremsstrahlung and characteristic x-rays are produced in this process. Also included in the simulation is the inherent filtration of a typical tube, 1mm beryllium and 1mm aluminium as consistent with the standard given in the IPEM report 78 (179). Photons exiting the tube, downward in this case, are scored and recorded in a phase space file. A graphical representation is given in Figure 10.2.
Figure 10.2: Geometry of a simple X-ray tube including tungsten anode and inherent filtration by 1 mm Beryllium and 1mm Aluminium.

A more elegant simulation would include the full geometry of an imaging process: the x-ray tube, added filtration, focus-to-object distance, contrast phantom, and the image receptor. Unfortunately, the efficiency of such a simulation would be prohibitively low. Figure 10.3 is a diagram of such a set-up. Here, a geometry has been programmed into the BEAMnrc code that omits only the image receptor.
Figure 10.3: Geometry of a Monte Carlo simulation of X-ray image formation using BEAMnrc.

The generation of X-rays by accelerated electrons has been included.

For the sake of this example, 3 scoring planes have been included to illustrate the amount of computation required for a robust simulation. The first scoring plane occurs at Z=0.5cm, just prior to the first layer of filtration. This records a phase space file containing the photons emitted directly from the x-ray tube in a downward direction. This is expected to contain a significantly higher count of particles than the subsequent scoring planes. As a note, in this simulation the option to activate ‘directional Bremsstrahlung splitting’ has been turned on. This creates multiple copies of photons that are produced by Bremsstrahlung effect. The option improves computational efficiency with insignificant sacrifices to statistical noise. The second phase space file has been designated at z=9.5cm, immediately following collimation of the X-ray beam by lead leaves. Here the number of photons has been reduced by the inherent filtration of the x-ray tube (Beryllium and Aluminium), and only those diverging
over an angle spanning approximately 15° will be allowed to pass through the lead jaws of
the collimator. The third scoring plane occurs at Z=100, recording the number of photons
transmitted through the contrast phantom. Again, a significant proportion is to be attenuated
by the soft tissue used in the simulation, lowering the count that are available to deposit dose
in the image receptor.

In a sample simulation run for one hour with these settings, the computation of
roughly five million histories was completed. The quantity of photons present in each phase
space file is shown in Table 10.1. Of the 5 million electrons simulated, only approximately
ten thousand photons will be generated that travel to the surface of the phantom. That is one
out of every 500. By simulating the X-ray tube separately and then using the spectrum to
generate a beam of photons emitted over the area of the surface, one can increase the
efficiency of such a simulation 500 fold. Improvements are even greater when one considers
that the calculation of characteristic and Bremsstrahlung X-ray formation by accelerated
electrons are two of the least efficient tasks that can be asked of the EGS code.

<table>
<thead>
<tr>
<th>Plane</th>
<th># of photons present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>173,527</td>
</tr>
<tr>
<td>2</td>
<td>9,617</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
</tr>
</tbody>
</table>

Table 10.1: Count of photons in each scoring plane for the simulation of 5 million electrons
incident upon the anode of an X-ray tube.

Though it may be desirable to design an X-ray simulation as a single step comprised
of X-ray generation, attenuation, and image formation, it is clearly impractical. At a rate of 85
histories per hour scored at the image receptor, a statistically reliable simulation (in the order
of 100 million photons at the IR pixels) would take over 100 years on a single PC!
10.4 Analysing simulated images from dosxyz *.3ddose files

The dose-scoring component used for the Monte Carlo simulations in this research (dosxyznrc) outputs data in the form of .3ddose files. These files are comprised of three sections: A header which describes the relative position of the voxels (in the case of the images shown in this example, they are distributed between -5.0 and +5.0 centimetres on the x- and y- axes with a single value [0,0.08cm] along the z), a listing of the dose values for each voxel (read out sequentially from x-min to x-max for each row along the y-axis, starting at y-min. For multiple depth values on the z-axis, each plane of x- and y- voxels is reported as stated above and then repeated for z=2, z=3 … up to z=max), and a listing of uncertainty values for each voxel (reported in the same order as dose values). For the sake of these
10. Appendices

experiments, only the dose values are of interest. The dimensions in the header are known from the input parameters, and we can estimate the statistical uncertainty in the images based on the standard deviation in the background voxels. As such, a convenient method of separating the relevant data from the extraneous values is necessary, especially when dealing with a large number of .3ddose files. Compounding this problem, data values are output as rows with each dose separated by a space and composed of (usually) four values. This data could easily be copied into a spreadsheet (such as Microsoft Excel) and by selecting a [space] delimiter each entry would be assigned to a separate column. That does not, however, address the issue of reorganising the rows and columns into the appropriate dimensions to replicate their special arrangement in the simulation (in the case of most simulations in this research: a 100 x 100 array).

Using mat lab, the data can be imported without the header rows (usually the first 36 rows) and a simple script can be written to shift each row into the proper alignment (collecting the first 25 rows from the .3ddose file into the first row as it appears in the image matrix, and so on). Occasionally, however, dosxyznrc will round off the final decimal digits in such a way that 5 dose values fit on a single line (this is more severe with noisy data sets when the uncertainty of some dose values exceeds 50% causing dosxyz to automatically zero of the value to a short “0.”). When this type of file is imported into Matlab, the 5th value is automatically shifted to a new row, followed by three NaN (“not a number”) values. A script that shifts rows automatically won’t be able to account for the added matrix size. The three NaN values appear as a distinct black cluster on the image and, more importantly, all the subsequent rows are shifted by 3 pixels causing a misalignment in the remaining portions of the image. The following approach has been an efficient remedy to the rearranging and removing ‘NaN’ problems.
Before importing the .3ddose file into Matlab, it has been shown most efficient to remove the non-relevant data (header and uncertainty sections). This can be quickly done in a text editor such as VIM or notepad. Generally any number that isn’t in the range of 10-12 to 10-16 is unwanted, and they are all grouped together into distinct sections at the start and end of the .3ddose file.

After cropping the unwanted values, import the data into Matlab and allow the software to create a new matrix variable. To use the script below, rename the data matrix “a” (without quotes), create a file called arrange5by5.m file in your working directory with the code described below, and run the command “arrange5by5” (named for the 5cm half widths on the x- and y- axes):

```matlab
b=reshape(a',[],1)
c=b(finite(b))
d=reshape(c,100,100)
```

Although the code is short, it accomplishes three key steps and with small modification can rearrange any matrix regardless of size. In this case, the script first converts the matrix from a size of approximately 2500 rows by 4 columns (if using the 100 by 100 pixel dimensions in similar image simulations) into a new matrix “b” that contains a single column with ~10,000 rows (a few extras values due to NaN may persist). The unwanted NaN values are removed in the formation of a third matrix “c” which copies “b” but only while referencing the finite values it contains (ignoring the NaNs). Finally a 3rd step rearranges the single-column data set into an appropriately-sized matrix “d” (in this case 100 x 100) that can be renamed accordingly. This same technique can be used when importing dosxyz files with different dimensions or describing multiple voxels in the Z-axis. One must simply adjust the output dimensions of the ‘reshaped’ matrix.
Data analysis requires the selection of regions-of-interest relevant to either contrast media or background. This script (analyse5by5) was written to quickly select ROIs and calculate their associated mean and standard deviation for the images from chapter 6 Optimisation of Au Contrast Enhancement by Monte Carlo Simulation. This reported the mean and standard deviation for regions corresponding to Au, Au+bone, bone, I+bone, & I. [Analyse5by5.m]

\[
\begin{align*}
\text{mm} & = \text{mean(reshape(d(2:15,22:27),[],1)) mean(reshape(d(2:15,40:45),[],1))} \\
& \quad \text{mean(reshape(d(2:15,48:53),[],1)) mean(reshape(d(2:15,56:61),[],1))} \\
\text{mean(reshape(d(2:15,74:79),[],1))} \quad \text{mean(reshape(d(23:36,22:27),[],1))} \\
& \quad \text{mean(reshape(d(23:36,40:45),[],1)) mean(reshape(d(23:36,48:53),[],1))} \\
& \quad \text{mean(reshape(d(23:36,56:61),[],1)) mean(reshape(d(23:36,74:79),[],1))} ; \\
\text{mean(reshape(d(44:57,22:27),[],1)) mean(reshape(d(44:57,40:45),[],1))} \\
& \quad \text{mean(reshape(d(44:57,48:53),[],1)) mean(reshape(d(44:57,56:61),[],1))} \\
& \quad \text{mean(reshape(d(44:57,74:79),[],1))} \quad \text{mean(reshape(d(65:78,22:27),[],1))} \\
& \quad \text{mean(reshape(d(65:78,40:45),[],1)) mean(reshape(d(65:78,48:53),[],1))} \\
& \quad \text{mean(reshape(d(65:78,56:61),[],1)) mean(reshape(d(65:78,74:79),[],1))} ; \\
\text{mean(reshape(d(86:99,22:27),[],1)) mean(reshape(d(86:99,40:45),[],1))} \\
& \quad \text{mean(reshape(d(86:99,48:53),[],1)) mean(reshape(d(86:99,56:61),[],1))} \\
& \quad \text{mean(reshape(d(86:99,74:79),[],1))} \\
\text{sd} & = \text{std(reshape(d(2:15,22:27),[],1)) std(reshape(d(2:15,40:45),[],1))} \\
& \quad \text{std(reshape(d(2:15,48:53),[],1)) std(reshape(d(2:15,56:61),[],1))} \\
& \quad \text{std(reshape(d(2:15,74:79),[],1))} ; \quad \text{std(reshape(d(23:36,22:27),[],1))} \\
& \quad \text{std(reshape(d(23:36,40:45),[],1)) std(reshape(d(23:36,48:53),[],1))} \\
& \quad \text{std(reshape(d(23:36,56:61),[],1)) std(reshape(d(23:36,74:79),[],1))} ; \\
& \quad \text{std(reshape(d(44:57,22:27),[],1)) std(reshape(d(44:57,40:45),[],1))} \\
& \quad \text{std(reshape(d(44:57,48:53),[],1)) std(reshape(d(44:57,56:61),[],1))} \\
& \quad \text{std(reshape(d(44:57,74:79),[],1))} ; \quad \text{std(reshape(d(65:78,22:27),[],1))} \\
& \quad \text{std(reshape(d(65:78,40:45),[],1)) std(reshape(d(65:78,48:53),[],1))} \\
& \quad \text{std(reshape(d(65:78,56:61),[],1)) std(reshape(d(65:78,74:79),[],1))} ; \\
& \quad \text{std(reshape(d(86:99,22:27),[],1)) std(reshape(d(86:99,40:45),[],1))} \\
& \quad \text{std(reshape(d(86:99,48:53),[],1)) std(reshape(d(86:99,56:61),[],1))} \\
& \quad \text{std(reshape(d(86:99,74:79),[],1))} \\
\text{mm2} & = 10e14*\text{mm} \\
\text{sd2} & = 10e14*\text{sd}
\end{align*}
\]

The mean values of each ROI are contained in a (5x5) matrix mm2 while the standard deviation values are included in sd2. The dose values of these matrices have been scaled by \(10^{14}\) in order to be imported into Microsoft Excel. The floating point precision of Excel isn’t sufficient to register the small Gy per history values output by dosxyz (~\(10^{-15}\)). This scalar cancels when computing contrast-to-noise ratio, but has been noted when reporting contrast (\(\Delta I\)) or noise (\(\sigma\)).
10. Appendices

10.5 Statistical Analyses of Image Data

In order to evaluate the significance of image contrast measurements from radiographic images, statistical analyses have been applied. The reports of significance beyond a critical $\alpha$ value are given in the respective chapters; however the detailed output of each test is described below:

10.5.1 Chapter 2

Independent measures, two-sample t-test values for CR (projection images) of equimolar CM samples at tube potentials between 40 & 80 peak kilovolts. Comparison of CNR values for CM regions-of-interest (n=18 per sample):

### t-Test: Two-Sample Assuming Equal Variances

<table>
<thead>
<tr>
<th></th>
<th>40 kVp</th>
<th>50 kVp</th>
<th>60 kVp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AuNP</td>
<td>Iodine</td>
<td>AuNP</td>
</tr>
<tr>
<td>Variance</td>
<td>0.414132</td>
<td>0.077903</td>
<td>0.378104</td>
</tr>
<tr>
<td>Observations</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>0.246018</td>
<td>0.2851</td>
<td>0.467184</td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>df</td>
<td>34</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>t Stat</td>
<td>27.53512</td>
<td>14.7525</td>
<td>7.620628</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>3.95E-25</td>
<td>1.21E-16</td>
<td>3.72E-09</td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.690924</td>
<td>1.690924</td>
<td>1.690924</td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>7.89E-25</td>
<td>2.42E-16</td>
<td>7.43E-09</td>
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<tr>
<td>t Critical two-tail</td>
<td>2.032244</td>
<td>2.032244</td>
<td>2.032244</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>70 kVp</th>
<th>80 kVp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AuNP</td>
<td>Iodine</td>
</tr>
<tr>
<td>Mean</td>
<td>8.145456</td>
<td>6.84359</td>
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<tr>
<td>Variance</td>
<td>0.346899</td>
<td>0.28623</td>
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<tr>
<td>Observations</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>0.316564</td>
<td>0.295079</td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
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<td>0</td>
</tr>
<tr>
<td>df</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>t Stat</td>
<td>6.941551</td>
<td>4.377415</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>2.65E-08</td>
<td>5.42E-05</td>
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<tr>
<td>t Critical one-tail</td>
<td>1.690924</td>
<td>1.690924</td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>5.3E-08</td>
<td>0.000108</td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.032244</td>
<td>2.032244</td>
</tr>
</tbody>
</table>
Independent measures, two-sample t-test values for CT images of equimolar CM samples at tube potentials between 80 & 140 peak kilovolts. Comparison of CNR values for CM regions-of-interest (n=21 per sample):

<table>
<thead>
<tr>
<th></th>
<th>80 kVp</th>
<th>100 kVp</th>
<th>120 kVp</th>
<th>140 kVp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AuNP</td>
<td>Iodine</td>
<td>AuNP</td>
<td>Iodine</td>
</tr>
<tr>
<td>Mean</td>
<td>97.54403</td>
<td>91.64815</td>
<td>150.4172</td>
<td>101.5747</td>
</tr>
<tr>
<td>Variance</td>
<td>69.66577</td>
<td>93.86913</td>
<td>133.0046</td>
<td>97.33352</td>
</tr>
<tr>
<td>Observations</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>81.76745</td>
<td>115.169</td>
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<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>df</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.020455</td>
<td>4.55E-18</td>
<td>4.55E-18</td>
<td>4.55E-18</td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.683851</td>
<td>1.683851</td>
<td>1.683851</td>
<td>1.683851</td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.021075</td>
<td>2.021075</td>
<td>2.021075</td>
<td>2.021075</td>
</tr>
</tbody>
</table>

Independent measures, two-sample t-test values for mean CNR values from CT images (n=11 per sample) and projection radiographs (n=12 per sample). CM samples were recorded at equal density (120 mg/mL) with a tube voltage of 120 kVp:
10. Appendices

### t-Test: Two-Sample Assuming Equal Variances

<table>
<thead>
<tr>
<th></th>
<th>AuNP</th>
<th>Iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>146.5667836</td>
<td>93.12098</td>
</tr>
<tr>
<td>Variance</td>
<td>394.4229165</td>
<td>56.84449</td>
</tr>
<tr>
<td>Observations</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>225.6337011</td>
<td>1.218095</td>
</tr>
</tbody>
</table>

#### Computed Tomography (120 kVp)

<table>
<thead>
<tr>
<th></th>
<th>AuNP</th>
<th>Iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>df</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>t Stat</td>
<td>8.344359598</td>
<td>2.912364</td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.00000003</td>
<td>0.004036</td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.724718218</td>
<td>1.717144</td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>6.04175E-08</td>
<td>0.008073</td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.085963441</td>
<td>2.073873</td>
</tr>
</tbody>
</table>

#### Computed Radiography (120 kVp)

<table>
<thead>
<tr>
<th></th>
<th>AuNP</th>
<th>Iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothesized Mean</td>
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<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>9.998309</th>
<th>11.31054</th>
</tr>
</thead>
<tbody>
<tr>
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<td>12</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>1.696658</td>
<td>1.696658</td>
</tr>
</tbody>
</table>

#### 10.5.2 Chapter 5

AuNP CM samples have been compared for difference in mean CNR values for data grouped over all tube potentials (40, 60, 80, & 100 kVp) by one-way repeated-measures ANOVA test. Each data point, CNR value, corresponds to the mean calculated from three images (n=3). The four AuNP groups (25 nm spheres, 30x60nm nanorods, 5.8nm spheres, & 3.8 nm spheres) are compared for significant difference in mean CNR and pairing (trend of change in contrast enhancement with adjustment of tube potential).

### Table Analyzed

<table>
<thead>
<tr>
<th>Repeated Measures ANOVA</th>
<th>CNR Gold Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td>0.6015</td>
</tr>
<tr>
<td>P value summary</td>
<td>ns</td>
</tr>
<tr>
<td>Are means signif. different? (P &lt; 0.05)</td>
<td>No</td>
</tr>
<tr>
<td>Number of groups</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>0.651878</td>
</tr>
<tr>
<td>R squared</td>
<td>0.178505</td>
</tr>
</tbody>
</table>

| Was the pairing significantly effective? | 0.999728 |
| R squared                            | 13430.1  |
P value < 0.0001
P value summary ***
Is there significant matching? (P < 0.05) Yes

<table>
<thead>
<tr>
<th>ANOVA Table</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (between columns)</td>
<td>0.223942</td>
<td>3</td>
<td>0.074647</td>
</tr>
<tr>
<td>Individual (between rows)</td>
<td>4613.69</td>
<td>3</td>
<td>1537.9</td>
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<tr>
<td>Residual (random)</td>
<td>1.0306</td>
<td>9</td>
<td>0.114511</td>
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<tr>
<td>Total</td>
<td>4614.94</td>
<td>15</td>
<td></td>
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</table>