Energy Efficacy of Gold Nanoparticles as Contrast Agents in Dual Energy Micro-CT

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Abstract

Micro-Computed Tomography (micro-CT) has been widely used as a non-invasive and high-resolution imaging modality in preclinical research. However, soft tissues (e.g. tumors) cannot be well distinguished because their densities are similar. To solve this problem, Gold Nanoparticles (AuNPs) are adopted as promising contrast agents since they are high atomic weight contrast agents and can be passively accumulated at the tumor sites. The aim of this study is to evaluate the efficacy of AuNPs as contrast agents using different energy x-rays for an in vivo study. An immune-compromised athymic nude mouse bearing a subcutaneous xenograft model of human lung cancer was imaged by a dual energy micro-CT with circular cone-beam geometry. The selected energies were 45 kVp and 65 kVp. 10µg AuNPs (200 µg/ml concentration) approximately 12 nm in size were injected subcutaneously into the tumor. The mouse was imaged at 0 hour, 3 hours and 24 hours post-injection. Images were reconstructed by the popular FDK algorithm. Our results show that AuNPs provided obvious greater contrast using 45 kVp-x-rays at 0 hour and AuNPs were not clearly shown in the images after 3 hours and longer. The raw datasets provided in this paper can be applied to evaluate other CT reconstruction algorithms and medical image analysis methods.

ABBREVIATIONS

AuNPs: Gold Nanoparticles; DECT: Dual-energy Computed tomography

INTRODUCTION

Lung cancer, including small cell lung cancer (nearly 20%) and non-small cell lung cancer (nearly 80%), is the second common cancer in both men and women. The American Cancer Society’s estimates for lung cancer in the United States for 2016 are about 224,390 new cases (117,920 in men and 106,470 in women) and 158,080 deaths (85,920 in men and 72,160 in women). The five-year survival rate for lung cancer is 54% for cases detected when the disease is still within the lungs. Unfortunately, most lung cancers have already spread widely when they are first diagnosed and the five-year survival rate is only 4% at an advanced stage [1].

Small-animal imaging plays a critical role to understand the mechanisms of disease [2] and Micro-Computed Tomography (micro-CT) provides non-invasive three dimensional (3D) imaging capabilities at low cost [3]. In preclinical studies, animals can be non-invasively imaged in vivo multiple times with micro-CT, which remarkably avoids ex vivo analysis, to some extent [4]. Meanwhile, the dual energy CT (DECT) can scan the subjects with two different x-ray spectra, and two sets of x-ray projections can be acquired from the corresponding x-ray spectra. Because the photoelectric effect in high atomic weight materials is highly dependent on x-ray energy, the attenuation coefficients of high atomic weight materials (e.g. bone, metal) would associate with the energy spectrum of the incident x-rays.

However, micro-CT cannot perform well in soft tissues due to their inherent low contrast resolution [4]. To solve this problem, exogenous high atomic weight and non-toxic contrast agents can be employed to enhance the contrast resolution [5]. Among those contrast agents, gold nanoparticles (AuNPs) are multifunctional, easily synthesized and bio compatible nanomaterials with significant therapeutic and diagnostic potential [6], and they have been reported to be non-toxic to human cells [7]. Therefore, AuNPs are excellent materials for x-ray contrast. Compared to the well-known iodine, gold provides about 2.7 times greater contrast per unit weight on account of its high atomic number and high photoelectric photon absorption [8]. With the
rapid development of nanoparticle contrast agents, various applications in small animal imaging have been reported. For example, AuNPs have higher photoacoustic contrast than mouse tissue ex vivo and can be visualized in mice in vivo following subcutaneous administration using photoacoustic tomography (PAT) [9]. AuNPs also have been used for passive tumor targeting in mice of brain cancer [10].

The effect of AuNPs as contrast agents is not only related to their properties (e.g., shape, size and concentration) but also dependent on the scanning conditions. Concentration of contrast agent is an essential determinant of attenuation and the attenuation value is linear with concentration up to 500-1000 mM (milli-mol/L). X-ray tube voltage is another major determinant of contrast and the attenuation of gold is maximal at 120 kV [11]. In the x-ray source, the amount of kinetic energy of each electron from the cathode is proportional to the tube voltage. When an electron strikes the target, a part of the electron kinetic energy is converted to x-ray photon energy due to Bremsstrahlung radiation and characteristic radiation. Although 120 kV is the optimal scanning condition, it is too high for in vivo small animal imaging.

Therefore, it is crucial to find the optimal energy of x-ray for in vivo small animal imaging research. In this study, we explore the energy effect of AuNPs in in vivo small animal for DECT imaging, because the DECT has the potential to be a particularly powerful modality with targeted contrast agents (e.g., AuNPs) and it can be used to separate AuNPs accumulated within soft-tissue sarcomas [12]. This data paper presents a mouse dataset acquired from a dual-energy micro-CT, along with the preliminary results for the evaluation of energy effect of AuNPs. The raw datasets can also be widely applied to evaluate CT reconstruction algorithms and medical image analysis methods.

The organization of the paper is as follows. The next section describes the in vivo experiment and the datasets, including data acquisition, data format, data pre-processing and image reconstruction. The third section presents the experimental results and analysis. The last section discusses the limitation of this experiment and future perspectives, and draws conclusions.

MATERIALS AND METHODS

Micro-CT

A small animal radiation research platform (SARRP, Xstrahl Ltd. United Kingdom) was used in this study (Figure 1). This facility combines cone beam CT (CBCT) imaging with a micro irradiation system, which can generate x-rays up to 225 kV. Cone beam scan can be achieved by rotating the horizontal animal bed between the stationary x-ray source and the 204.8x204.8 cm² flat panel CsI (TI) scintillation detector which is composed of 1024x1024 detector cells. In this study, a circular cone-beam scanning geometry was used with an angular step of 1°. That is, 360 projections can be acquired for a full scan. The field of View (FOV) of this facility is 10 cm in diameter at the isocenter. The radiation dose is 1 Gy to 3 Gy for one full scan, and the full-scan imaging time is less than 4 minutes for combined data acquisition and reconstruction.

Gold nanoparticle and Mouse

The subcutaneous xenograft model of human lung cancer was used in this study. Human lung cancer cell line, A549 cells (from ATCC) were subcutaneously implanted into the left flank of an immune-compromised athymic nude mouse. Two weeks later, the tumor was developed with a size of about 3 mm in diameter. To suppress the motion artifacts during the dual energy micro-CT scan, before the start of the experiment, the mouse was first anesthetized by isoflurane that is a nonflammable, nonexplosive inhalation anesthetic. Then, it was put on the animal bed of the micro-CT scanner.

Data Acquisition

Before the mouse experiment, two groups of white field
projections were acquired using the SARRP with a circular full-scan mode at 45kVp and 65kVp for the preprocessing step to linearize the projections. They were respectively named as ‘WhiteFieldImage_45kVp.raw’ and ‘WhiteFieldImage_65kVp.raw’. Here, the “white field image” means that the projections were acquired with the x-ray on but no subject in the field of view (FOV). Then, the anesthetized mouse was put on the animal bed, two separate groups of projections were acquired at 45kVp and 65kVp, and they were respectively named as “ProjectionImage_45kVp_NoAuNP.raw” and “ProjectionImage_65kVp_NoAuNP.raw”. After that, 10µg AuNPs (200 µg/ml) approximately 12 nm in size were injected intratumorally, and the x-ray source was turned on to scan the mouse on different time points to evaluate the energy efficiency of the AuNPs. For each time point after the injection of AuNPs (e.g. 0 hour, 3 hours, 24 hours), two groups of projections were also acquired, and they were respectively named as, “ProjectionImage_45kVp_AuNPs_TimePoint.raw” and “ProjectionImage_65kVp_AuNPs_TimePoint.raw”, where “TimePoint” means 0 hour, 3 hours and 24 hours. For all the acquired datasets, the cone-beam circular geometry was utilized and the corresponding FOV fully covered the cross-section of the mouse. The x-ray source of SARRP was filtered by 1.0 mm Aluminum, the mAs per projection was 0.082mAs (0.5mA*59s/360) the ideal detector plane was parallel to x-y plane, and its center was collinear with the focal spot and the center of rotation. In this experiment, the center of the detector slightly shifted away from the center of the ideal detector plane which was corrected by image reconstruction algorithm. The scanning parameters and imaging geometry are summarized as in Table 1.

### Methods for Data Loading

In this data paper, six groups of datasets are provided in *.raw format, which are ‘WhiteFieldImage_45kVp.raw’, ‘WhiteFieldImage_65kVp.raw’, “ProjectionImage_45kVp_NoAuNP.raw”, “ProjectionImage_45kVp_AuNPs_TimePoint.raw”, “ProjectionImage_65kVp_NoAuNP.raw”, “ProjectionImage_65kVp_AuNPs_TimePoint.raw”. Their corresponding calibration files are in *.cal format which include the scan parameters mentioned in Table 1. Raw format datasets can be loaded using MATLAB. The ‘fopen’ and ‘fread’ commands can be used to load the raw projections into the workspace. The ‘fopen’ and ‘fread’ commands can be used to load the raw projections into the workspace. The ‘fread’ command can be first used to import the projection image as a 1D column vector. Then, the ‘reshape’ command can be used to reorganize the data column-wise to form a 3D matrix.

### Projection Preprocessing

Before the preprocessing step for the raw projections, the white field projections were averaged to be used as the incident intensities corresponding to each x-ray path. Let \( I_0 \) represent the incident x-ray intensity. As the aforementioned, before the injection of AuNPs, the mouse was scanned using 45kVp and 65kVp simultaneously. After the injection of AuNPs, the mouse was first scanned immediately using 45kVp and 65kVp. Then, it was scanned again at 3 hours and 24 hours post-injection, respectively. As a result, there were 8 groups of projection images (I). Those projection images were preprocessed for linearization before image reconstruction.

According to the Beer-Lambert law, the linear relationship between absorbance and concentration of an absorbing species can be modelled as

\[
\ln(I) = A \times \mu x + B
\]

where \( \mu \) means attenuation coefficient of the \( k \)th absorbing species, and \( \chi \) means the thickness of the \( k \)th absorbing species. Because \( I_0 \) is unknown, a separate experiment was performed to measure \( I_0 = I_0^\alpha \), where \( \alpha \) is a magnification factor due to different mAs. The linearized projection images can be given by

\[
I_{opt} = \sum_k \mu_k \chi_k = -\ln(I_0) + A \times \ln(I_0) - B
\]

where \( I_{opt} \) is the linearized projection images, and \( B \) is a constant corresponding to the magnification factor \( \alpha \). Because Eq.(1) is only valid for monoenergetic x-ray source and the real micro-CT system employs a polyenergetic x-ray source, an additional constant \( A \) is introduced in Eq.(2) to compensate for this effect. The original projections images are fitted to find the optimal parameters \( A \) and \( B \) for the best linearization (Figure 2). Using the least square method, the optimal constants were fitted as \( A=2.0 \) and \( B=\SI{3.11}{\text{at 45kVp}} \), and \( A=2.0 \) and \( B=\SI{-1.88}{at 65kVp} \).

In this study, the raw projection image \( (I) \) is a three-dimension matrix and \( \text{size} \) is 1024 (the total number of horizontal detector cells)×1024 (the total number of vertical detector cells)×360 (the total number of projection views). After optimizing, removing the bad pixels and merging detector cells, we obtained 8 series of projection images in *.mat format. Then, all images were reconstructed by the well-known FDK algorithm.

### The FDK Algorithm

In this study, the conventional FDK algorithm [13] was employed to reconstruct 3D images. Specifically, in the equal-distant geometry, the FDK algorithm was given by:

\[
f(x) = \frac{1}{Z^3} \int_{-\infty}^{\infty} \frac{D^2(u, \lambda)}{D(u, \lambda)} \int_{-\infty}^{\infty} \left[ g(u, v, \lambda) \frac{D(u, \lambda)}{\sqrt{D^2(u, \lambda) + v^2}} \right] \delta(\xi - \lambda u) \delta(\eta - \lambda v) d\xi d\eta \]

Where \( g(u, v, \lambda) \) represent the linearized projection data, \( \lambda \) is the angular position of the current x-ray projection, \( (u,v) \) is local coordinate on the detector plane, \( h(u) \) is the ramp filtering kernel function along the horizontal direction, \( D_u \) is the source-to-detector distance, \( D \) is the distance from the x-ray source to the imaging pixel \( X \) to be reconstructed, and \( \delta(\xi - \lambda u) \delta(\eta - \lambda v) \) is the local coordinate in the detector plane of the projection position of the ray passing \( X \). This algorithm was implemented in three steps:

### Table 1: The Scanning Parameters and Imaging Geometry.

<table>
<thead>
<tr>
<th>Scanner</th>
<th>Source to Detector Distance (mm)</th>
<th>Detector to Origin Distance (mm)</th>
<th>Number of Projection Images</th>
<th>Projection Image size (Pixel)</th>
<th>Projection Image size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARRP</td>
<td>350.721</td>
<td>189.849</td>
<td>360</td>
<td>1024×1024</td>
<td>204.8×1024</td>
</tr>
</tbody>
</table>


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(a) For each measured data \( g(u,v,\lambda) \), it was pre-weighted by a cosine factor \( \sqrt{D_D^2 + u^2 + v^2} \);

(b) For each view \( (\lambda) \), ramp filtering was performed row by row on the weighted data;

(c) The image was finally reconstructed by back projecting the filtered data with a weighting function \( \frac{(D_D^2 + u^2 + v^2)D}{D^2D_D^2} \).

**EXPERIMENTAL RESULTS AND ANALYSIS**

After the injection of AuNPs, the mouse was scanned at 0 hour. There constructed volumetric image showed the significant effect of AuNPs (200 µg/mL concentrations) at two energy levels of 45kVp and 65kVp. Moreover, the AuNPs provided greater contrast especially in 45kVp energy (Figure 3). After 3 hours and 24 hours, the AuNPs distributed throughout the whole body of the mouse. As a result, they were not clearly shown in the images (Figure 4). A rule of thumb is that the average energy would be 1/3 of the peak value without filter. In this experiment, 13 mm Aluminum filter was used. For the energy level 45kVp, the average energy of the beam is approximate 28keV, and the mass attenuation coefficient \( (\mu / \rho) \) of gold (~37.782cm²/g) is much higher than that of the soft tissue (~0.468cm²/g) due to the fact that gold is a high-Z material. For the energy level 65kVp, the average energy of the beam is approximate 35keV, and the mass attenuation coefficient \( (\mu / \rho) \) of gold (~20.25 cm²/g) is still higher than that of the soft tissue (~0.324 cm²/g) but the difference between gold and soft tissue is smaller comparing with that at the energy level 45kVp. Thus, the AuNPs provide greater contrast using x-ray with lower energy.

From Figures 3 and 4, we can see that there were no obvious contrast improvement after 3 hours and 24 hours. The concentration of injected AuNPs (200 µg/mL concentrations) is the primary reason, although the effect of AuNPs is obvious at 0 hours. A previous study on high-z agents (Iodine) showed that great contrast images can be obtained at a concentration of 100 µg Au/mL [14]. There is another research used the concentration of 270 mg Au/mL. Quantitative pharmacokinetics using graphite furnace atomic absorption spectroscopy showed that the highest blood gold concentration 15 min after injection was in the kidney, about 10.6% id/g (percent of the injected dose per gram of measured tissue), followed by tumor, about 4.2% id/g; and tumor at 24 hours retained more than half of its value reached at 15 min. [8]. In this study, the concentration of injected AuNPs is much smaller than 270 mg/mL. After 3 hours, the concentration of retention can’t be differentiated by a micro-CT.

**DISCUSSIONS AND CONCLUSIONS**

Compared to conventional single energy CT, the DECT can provide additional diagnostic information using material decomposition technique. Within one voxel, there are two reconstructed attenuation values corresponding to two different energies. The mathematical decomposition of multiple materials depends on having high quality measurements of attenuation at each voxel [4]. The attenuation coefficients among these materials must be distinguishable. For energies below 80keV, because the attenuation coefficient \( (\mu / \rho) \) of gold is much close to that of bone, it is difficult to discriminate these two materials. Gadolinium (Gd) (K-edge: 50.2keV) is an alternative choice. Gadolinium chelator-
coated GPNPs could be used as contrast agents for both in vivo x-ray and MR imaging [15]. In general, micro-CT has lower SNR than clinical CT. Because of the radiation dose limitation for in vivo studies, the noise can’t be decreased by increasing the number of incident x-ray photons. Before the DE decomposition, applying joint domain bilateral filtration (an edge-preserving, smoothing filter that incorporates data from both energy sets) can significantly improve the DE decomposition accuracy, precision, and limitations of detectability [16]. Thus, the noise could be reduced by using appropriate image postprocessing algorithms. Hence, the provided raw datasets in this data paper can be widely applied to evaluate different image reconstruction algorithms and image processing methods, such as material decomposition and digital subtraction angiography techniques.

In this study, our experiment showed that AuNPs as contrast agents can improve the definition of the subcutaneous xenograft model of human lung cancer in a mouse by increasing photoelectric photon absorption. This could help to increase the accuracy of tumor diagnosis and staging and aiding volume and definition in radiotherapy planning [17]. Meanwhile, images were taken at various time points (0 hour, 3 hours and 24 hours) after the injection of AuNPs, and we observed that AuNPs did not concentrate inside the tumor after 3 hours. This is because they had already cleared through the kidney. To detect smaller tumor at staging and contribute to image-guided radiotherapy, sufficiently high concentration of AuNPs could be used to improve the retention time and contrast [18].

REFERENCES


Cite this article