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Time dependent variations in X-ray Computed Tomography data during repeated scanning

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Abstract

High resolution laboratory X-ray Computed Tomography (CT) systems are capable of providing three dimensional (3D) images at spatial resolutions ranging from hundreds of microns to better than 100nm. Increasingly such images are being used to quantify features (porosity, defects, geometry, phase fractions, etc) by segmenting the images into different regions on the basis of greyscale values. As a non-destructive technique X-ray CT is particularly well suited to time-lapse (3D+time) studies, however, temporal variability in both the source and or detector can alter the grey scale values of the reconstructed volumes. In this paper we examine the extent of the greyscale variation as a function of time. Our results show that detector performance is not constant over time and that decreasing source accelerating voltage and decreasing exposure times introduce greater variability. We examine the implications of temporal variations in contrast on the quantitative analysis of 3D images. Our work demonstrates the dangers of using global greyscale automatic thresholding and shows that a careful choice of thresholding technique, coupled with pre-experiment conditioning of detectors can minimise variability in quantities obtained at different times.
Introduction

X-ray Computed Tomography (CT) has rapidly become established as a day to day characterisation tool in materials science, for both academic studies and industrial measurements. Advances in instrumentation have resulted in laboratory X-ray CT instruments capable of spatial resolutions extending from hundreds of microns to better than 100 nanometres [1] with measurement times shortened by the advent of CCD area detectors and the level of 3D visualisation and analysis revolutionised by increases in computing power. These factors have enabled the widespread application of CT as a tool for materials analysis [2, 3].

The 3D quantification and analysis of internal features or phases within a specimen lies at the core of most CT investigations [4]. Early instruments were designed specifically for making very accurate quantitative measurements of bone mineral density from the recorded attenuation contrast. For quantitative densitometry it is important to make sure that the grey level fluctuation observed is only due to the change in material density rather than instrumental effects. Consequently calibration standards and procedures were developed to accurately relate the recorded grey level to the attenuation coefficient [5]. As pointed out in [4] in many ways today’s full field scanners are not as well suited for the quantification of the linear attenuation coefficient, which requires a well-defined source, the collection only of photons that travel in straight lines and a simple application of Beer’s law for attenuation. In trading discrimination for speed, modern scanners collect scattered photons, often employ white radiation such that Beer’s Law is not obeyed (giving beam hardening) while CCD systems are prone to uneven pixel responses and various time dependent effects.

Rather than use attenuation contrast (grey level) as a direct indicator of density the majority of studies now use changes in grey level to distinguish specific regions. In this approach a feature is distinguished and isolated by applying a threshold range based upon its greyscale values, allowing subsequent visualisation and characterisation of the feature in 3D. This allocation of pixels to phases or features according to grey level ranges, known as segmentation, has markedly reduced the use of calibration standards because absolute levels are not required to distinguish regions of interest. While this is in many cases sufficiently accurate it should be noted that the grey level histogram is usually composed of overlapping peaks. Consequently small changes in the thresholding range (selected either manually [6, 7] or automatically [8-12]) can result in significant variations in the volume and morphologies of the identified phases [4]. The extent to which such changes will affect the quantification is a function of the thresholding method chosen. At present there are no agreed standards regarding either the collection of X-ray CT data or data processing, although the ASTM has issued guides regarding both [13, 14]. The use of a specified threshold range over an entire data volume is known as global thresholding. Global thresholding is the easiest method of segmentation implementation and as such is the most popular method amongst CT researchers. Unfortunately CT images can display a number of artefacts such as a beam hardening [15, 16]. Because they affect the grey levels such artefacts can seriously affect the segmentation. Those grey level artefacts which
are long-range, such as beam hardening, can be partially accounted for using locally adaptive segmentation methods. Adaptive thresholding techniques segment features based upon the local grey scale change over a pre-defined local area, this approach takes into account any background changes that occur over larger volumes resulting in a more accurate feature segmentation and a review of thresholding techniques has been considered by others such as [10].

The absence of discrete greyscale level bands also means that comparing metrics obtained from images acquired on different instruments, even when the effective pixel sizes are the same, is fraught with difficulty because the greyscale distribution’s will be different. Even when comparing images obtained at different times on the same instrument under nominally the same conditions the acquired greyscale values are subject to temporal variations in a) the x-ray source and b) the variability in the pixel response with time for the detector. Though largely ignored such effects can be important either when comparing results collected at different times – for example as part of a quality control (assurance) process, or as part of a time-lapse sequence.

Changes in the source are usually considered to be negligible over time, however, there are a number of reasons why the source might vary over time. In the laboratory these could arise from thermal expansion induced movement of the target or variations in the electron beam that strikes it, or voltage instabilities (<1% for modern sources) or target degradation. For synchrotron sources this might arise from beam instabilities or drifts.

Numerous factors can cause a variation in the response of X-ray detectors over time. These will directly affect a detector’s maximum frame rate, energy and contrast sensitivity, and signal to noise ratio. Hence, temporal variation should be considered for specific detector systems, however, some issues will affect all detectors as they age. The most common problem is damage accumulated over months to years, manifest in the form of dead (non-responsive) pixels or defective ones whose behaviour differs from their neighbours. Most systems use calibrations to correct the resulting artefacts. Another practical problem encountered in CT is that of residual (ghost) images following exposure, caused by the time required for pixels to dissipate residual charge. This can cause significant artefacts in scans where the detector has been over exposed, but will dissipate with time, and can be easily avoided by adjusting scan settings in line with manufacturer’s guidelines. Another problem that impacts some detectors is a gradual accumulation of charge, directly altering the sensitivity. This can be avoided by discharging the detector at the end of each scan, effectively returning the system to a zero reference point. Finally, operating temperature can affect a detector’s performance; however modern detectors have a high degree of temperature stability and are usually housed within temperature controlled units.
This study investigates the instrumental and analytical issues that hamper the accuracy, reliability and robust quantification of objects overtime and aims to identify methods to minimise these effects on image quantification of a time lapse series. The two main causes of instrument variation are:

I. The X-ray detector - X-ray detectors are optimised for a specific energy range that is determined by its constituent energy sensitive materials. This results in detectors demonstrating a variability in response time related to the incident photon energy spectra produced by the X-ray source. In addition due to the charge produced by the incident photons, X-ray detectors are susceptible to charging over time which can directly affect the performance. In this study the detector exposure time during X-ray CT batch scans is investigated for three different energy settings.

II. The X-ray source – Changes in the emitted X-ray spectra will directly affect the X-ray detector response and consequently the grey scale values in the reconstructed X-ray CT scan. Changes in the X-ray source can be due to:

   a. Instability of the X-ray tube accelerating voltage
   b. Changes in the X-ray spot on the target material. This can be caused by heating of the target material over time due to poor cooling or degradation of target material due to damage
   c. Degradation of the X-ray filament over time results in a decreased photon count. This usually results in the application of a different accelerating voltage in order to achieve the appropriate transmission and or flux through the specimen, however this results in a change in the emitted X-ray spectra

In this study changes in the source variability have been minimised and the effect of the X-ray source upon the grey scale values in the reconstructed X-ray CT batch scans have been investigated.

Finally the study will investigate the extent to which the following two methods can be used to correct for variability in the grey scale histograms between X-ray CT batch scans:

   a) The application of reference images to correct for instrument variability and drift - Commercial CT systems are fitted with the ability to take black and white reference images, these are essential for the reconstruction stage. Some systems use the option of multiple reference images that can be taken between scans to account for changes in either the source or the detector. In the study the effect of reference images upon the grey scale histogram variation has been investigated as well as the time for discharging of the X-ray detector
   b) The extent to which the metrics obtained using different automatic segmentation strategies (global and locally adaptive) are sensitive to the changes in greyscale caused by the above effects.
Experimental

Scanning Experiments

By way of a simple test-object a single dried excised tibia bone from a C57BL/6 black mouse (Harlan Laboratories) was selected. Important clinical assessments (such as the efficacy of treatments in mitigating bone loss due to osteoporosis) are based on small yet significant changes in the volume and microstructure of bone within a region of interest CT scan [17-19]. Although many clinical studies are concerned with bone density measurements and hence absolute values of greyscale, the quantification of bone microstructure and morphology is often carried out on segmented images [20-22]. At a more general level, the sample is representative of many porous and foam structures evaluated by CT. The bone was mounted vertically on the end of an aluminium rod alongside two small Perspex and titanium samples to provide reference peaks.

The specimen was analysed using a Nikon 225/320 kV CT system housed within a customised bay with a 2k x 2k Perkin Elmer 1621-16-bit amorphous silicon flat-panel detector having 200 micron pixel pitch and an aluminium window (XRD 1621 AN3 CT grade), a 225kV X-ray source with a multi-metal reflection target (in all cases a tungsten target produced the best signal to noise ratio for the accelerating voltage of 40-60kV). 2001 projections were acquired over 360° with the specimen to X-ray source and specimen to detector distances of 34.2mm and 1007.0 mm respectively, resulting in a reconstructed voxel size of 6.8μm. To avoid over-exposing the detector, the current was adjusted to obtain an average photon counting rate of approximately 55000 counts per pixel per projection. For all scans a single reference/calibration image was used for each scan series. In addition, the focus was optimized for the low energy spectrum and the system alignment was rechecked in order to optimize resolution.

Experiment I: In order to assess the variability in data acquisition over time a series of measurements (scans) were made of the test-object, as might be done during a time-lapse experiment.

24hrs prior to the experiment the instruments was set with the specimen and focused at 50kV. The X-ray source (under vacuum) and detector were turned off for 24hrs to ensure no previous experiments could influence the results. Before each scan series:

- The X-ray source was conditioned at 225kV to ensure beam stability (+/- 1 kV).
- After beam conditioning the X-ray source was turned on and a lead shield placed in front of the X-ray source to avoid any incident photons on the detector. This precaution was taken to ensure that the X-ray spot on the target achieved an equilibrium size and shape. However it is noteworthy that experiments conducted without this step did not affect the results.
- A reference image was then collected and the scan series (Table 1) was then started.
At the end of each scan series the instrument source was turned off to minimise filament degradation and
the instrument was left for 7 hours before starting the above three steps for the next scan series (Table 1).

<table>
<thead>
<tr>
<th>Series Run No</th>
<th>Voltage /kV</th>
<th>Current /μA</th>
<th>Projection time / ms</th>
<th>Total number of scans</th>
</tr>
</thead>
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<td>50</td>
<td>80</td>
<td>2</td>
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</tr>
<tr>
<td>I.2</td>
<td>50</td>
<td>170</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>I.3</td>
<td>50</td>
<td>380</td>
<td>0.5</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 1: Instrumental parameters for experiment I. To maintain a good signal to noise ratio (55000 counts
per detector pixel) the filament current is increased as the projection acquisition time is decreased.

Experiment II: To assess the effect of accelerating voltage on the variability in image acquisition over time a
series of measurements (scans) were made of the test-object as might be done during a time-lapse
experiment (Table 2). The experimental procedure before each scan series was performed as for
experiment 1 using three different accelerating voltages.

<table>
<thead>
<tr>
<th>Series Run No.</th>
<th>Voltage /kV</th>
<th>Current /μA</th>
<th>Projection time / s</th>
<th>Total number of scans</th>
</tr>
</thead>
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<td>380</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>II.2</td>
<td>50</td>
<td>170</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>II.3</td>
<td>60</td>
<td>105</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2: Instrumental parameters for experiment II. To maintain a constant 55000 counts per detector pixel
for each series run, the filament current is decreased as the accelerating voltage is increased. This also
ensures that the detector is not over exposed during the experiment.

Experiment III: To determine the extent to which detector charging may contribute to variability in image
acquisition over time, and whether the application of new reference images may correct for this effect. For
this experiment the specimen was scanned at 50kV, 170 μA and a projection time of 1 second. A reference
image was taken at the start of the series was used for the first four scans that were separated by several
hours (during this time the X-ray source was turned off) to ascertain the effect of detector charging upon
image variability. Further scans were then collected using new reference images to determine whether the application of new reference images resulted in the reduction of image variability as is sometimes recommended by instrument manufacturers.

**Image segmentation methods**

Following image acquisition, the datasets were reconstructed using Nikon Metris CT-Pro (X-TEK CT Pro 3D Version XT 2.2 service pack 10) (see for example Figure 1b.) which utilises variant of the FDK reconstruction code [23]. Subsequently the grey scale populations were evaluated using Python scripts¹ written in-house (Figure 1a). A peak finding algorithm was then applied to the smoothed data in order to determine the location of the peaks corresponding to air/Perspex, bone and titanium respectively (Figure 1). The first script (implementing the ‘ctagg’ utility) converts the XCT scan data into an aggregated CSV file of greyscale counts. The specified scan is opened as a sequence of 2-dimensional numpy arrays and a tally of greyscale values is generated. This process iterates over the entire sequence and Python's inbuilt CSV writer is then used to write the contents of the counter to the specified output file. The outputted CSV data can subsequently be displayed as a histogram of frequency vs grey scale values of the voxels for each scan (Figure 1). A second Python script applies a moving average smoothing to the binned data (window size 5). A smoothing window of 5 was chosen as the optimal balance between noise reduction and peak resolution i.e. a larger window further reduces noise but compromises our ability to accurately locate the peak position. A peak finding algorithm was then applied to the smoothed data in order to determine the location of the peaks corresponding to air/Perspex, bone and titanium respectively.

In order to examine the robustness of the quantification over time in the face of any changes in the grey scale distribution five thresholding methods are compared.

**Trial 1: Global static minima thresholding.** Despite known limitations [4] global thresholding techniques are among the most commonly used segmentation methods largely due to ease of application and so are considered here. It is common practice to select the threshold values for discriminating between phases (in our cases air-bone and bone-titanium) in terms of minima in the greyscale distributions (Figure 1c). For this method a simple thresholding function using the SciPy signal processing package function in Python was used to locate the minima between the peaks in the intensity spectrum (the raw code can be found in the supplementary information). For each run this threshold range was determined from the first scan and then applied to all other scans in the series run. In this way the first scan acts as a reference point by which the variation in consecutive scans within the series run can be directly gauged against. This threshold technique is referred to here as the “static” method. This method produced an accurate segmentation of

¹ See supplementary information
the main bone section as shown in Figure 1c, however a detailed inspection of region 1 (Figure 1c) shows that the volume is slightly underestimated although some of the larger pores in this region are better defined. An inspection of region 2 (Figure 1c) shows that the segmentation technique was unable to characterise the fine trabecular structure when compared to the unsegmented image shown in Figure 1b. This shows that the global minima thresholding slightly underestimates the volume of bone particularly in the fine trabecular section (region 2).

Trial 2: The global variable minima thresholding method is performed as for method 1 described above. However the thresholding boundaries were determined for each dataset within the series run. This thresholding technique is commonly employed in X-ray CT for the identification of phases. This thresholding technique is referred to here as the “variable minima”.

Trial 3: Global thresholding based on mid-points between peaks. This method is illustrated in Figure 1a and the segmentation results are shown in Figure 1d. For this method a simple thresholding function using the SciPy signal processing package function in Python was used to locate the peaks in the intensity spectrum (the raw code can be found in the supplementary information). The midpoint was the calculated based upon these positions as shown in Figure 1a. This method is popular when the minima between peaks (method 1 above) are not well defined, which can cause repeatability issues relating to image noise. In cases where peaks are well defined this method can result in reduced variability in the thresholding. As for Method 1 the boundary conditions were found for the first scan and then applied to all other scans in the series run as a reference point. This thresholding technique is referred to as “static centre”. In this investigation this method produced a better segmentation of the fine trabecular structure (Region 2 Figure 1d) at the expense of over-estimating the main bone section (Region 1 Figure 1d) where many of the pores are no longer present when compared to the unsegmented original data in Figure 1b. A direct comparison of the number of pixels selected by the centre method verses the minima thresholding technique shows an increase of 3.35%.

Trial 4: The static centre technique described above was then applied to each data set with in a series run. This allows the thresholding boundaries to move with the peaks in cases where the main peaks are shifted due to a globally changing detector response. This thresholding technique is referred to as “variable centre”.

Trial 5: As summarised in [4] locally adaptive thresholding technique have certain advantages over global thresholding techniques. While the global thresholding techniques (methods 1-4 above) does not take into account the change in grey scale values between neighbouring voxels, the local thresholding techniques look for changes in gradient between neighbouring voxels over a specified range. By taking into account local changes in the greyscale values locally adaptive thresholding techniques are thus less prone to changes in absolute greyscale across the volume as might occur due to X-ray CT artefacts such as beam
hardening or the cone beam effect for example. The latter is shown in Figure 1e where this method shows a better segmentation of the fine trabecular structure (Region 2 Figure 1e) while still discriminating the pores in the main bone (Region 1 Figure 1d), although the latter are possible slightly over estimated when compared directly with the original segmented image (Figure 1b). A direct comparison of the number of pixels selected by the adaptive method verses the minima thresholding technique shows an increase of 4.05%. In this study the SciPy signal processing package function in Python was used to perform the thresholding. In this function a Gaussian fit was applied to the data and a kernel (or window) size of 60 pixels was applied.

Results and discussion

Experiment I: Thresholding variation for X-ray CT time-lapse studies

Figure 2a shows the temporal variation in grey scale distribution recorded for successive scans based on 1s projection acquisition times. A shift in the bone peak position to a lower grey scale value is evident with time being most marked for the first few scans. Similar trends occur for projection times of 0.5 and 2.0 seconds (Table 1). Changes in the magnitude and position of the bone peak directly impact upon the thresholded voxel count (Figure 2b), resulting in 0.6-1.5% variation in the estimated bone volume between scans settling down after 90mins or so.

A repeat of experiment one procedure using only the conditioning step and reference image collection; before scanning showed similar results to those shown in Figures 2a & b. The lack of change when preparing the source indicates that the variation observed in Figure 2a for consecutive scans does not appear to be source related (spot size, shape etc.). This indicates that the origin of the observed variation is related to the X-ray detector behaviour and is fully discussed in the results section for experiment 2; where the X-ray detector behaviour is fully explained.

Experiment II: Variability as a function of accelerating voltage

Figure 3a shows greyscale histograms for data collected with the source operating at 40, 50 and 60kV. As the accelerating voltage increases, the bone and titanium peaks are shifted left, and narrowed. Unsurprisingly, with increasing voltage (higher mean energy) the transmission through the sample increases lowering the greyscale level corresponding to bone. In effect, the effective decrease in the greyscale range observed in Figure 3a results in an increase in the number of voxels at each grey scale value, explaining both the sharpening and increased height of the bone and titanium peaks. Effectively this changes the thresholded volume from $4.27e^7$ voxels to $4.09e^7$ (-4.5% change) and $4.16e^7$ (-6.2% change) voxels respectively when the accelerating voltage is increased from 40 to 50 and finally 60kV; this highlights
the quantitative problems that are introduced when the accelerating voltage is increased during in-situ studies.

It is clear from Figure 3b) that the grey scale variations over time lead to changes in the segmentation and that this appears to plateaux more quickly at lower voltages (~30mins at 40kV) compared to higher voltage (~100mins at 60kV). This can be explained as follows; when incident X-ray photons first interact with the detector (more specifically when the signal from the scintillator hits the amorphous Si detector array), electron–hole pairs are formed. The number of these produced is directly proportional to the incident photon energy and the number of photons. Electron-hole pairs then travel to the anode and cathode on the detector array where their presence is registered. The greyscale value from each pixel - which is proportional to the registered electrons on the cathode - is then recorded but charge dissipation of the detector is not 100% efficient over the acquisition time. The observed shifts appear to result from a gradual build-up of charge in the detector which ultimately approaches a dynamic equilibrium where the charge generated by the incident photons is equal to the charge dissipated at the cathode. The increased time required for the segmentation volumes to plateaux with increased accelerating voltage is likely linked to the associated increase in charge production within the detector. The above effect could be associated with heating of the detector over time, however this can be discounted due the X-ray source and detector unit being setup in a large walk-in chamber that is temperature controlled. In addition the actual energy that is incident upon the detector is below 0.5W of power and will have a negligible effect.

**Experiment III: Detector charging and application of reference images**

During the study the X-ray source has been carefully conditioned to minimise variation, while in experiments 1 & 2 the source of the voxel thresholding variation has been attributed to the gradual charging of the detector. Figure 4 shows that when large time periods are present between scans the variation in the thresholded volume (voxel count) is less than 0.3% for datasets collected seven hours apart using the same black and white reference images. This is far smaller than that observed during the continuous series scans shown in either experiments 1 and 2 (Figures 2b & 3b).

Scans after 24hr after the first scan had a new second reference image applied (Figure 4). The application of a new reference image resulted in a maximum change of 0.3%. Although this is significantly smaller than the change observed for the series scans (Experiments 1 & 2). These results demonstrate that the variability in voxel count stem predominantly from the detector, resulting from a build-up of charge. Crucially, despite large time spacing between scans (Figure 4), the remaining variability indicates that the time required for the charge on the detector to fully dissipate is longer than 12 hours. The use of a new reference image prior to a scan to account for changes in the detector or source do not appear to re-calibrate the images and therefore do not minimise the thresholding variation.
Impact of segmentation techniques

In previous sections, thresholds were calculated based on spectrum minima (as highlighted in Figure 1a) from the initial scan and held static throughout subsequent scans within each series. This method was used as a standard by which subsequent variation in the X-ray CT datasets could be compared. For well-defined histograms with few characteristic phases and little variance across the reconstructed volumes, such as the specimen used in this study, global thresholding is a quick and robust thresholding technique for the identification of the bone material or any other well defined two phase material. The use of the minima between material peaks as the thresholding boundary [24-26] is in theory a consistent method. However the variation in peak position detailed above will change these boundary conditions resulting in a changing voxel count over time.

To assess the impact of this experimental choice, Figure 5 illustrates the variation in voxel counts for the thresholding techniques described previously. The centre-based variable thresholding methodology provided a voxel count variation of < 0.4% between different scans within the same batch. This compares favourably with the static thresholding method, which has a maximum error of 1.4%. However, the minima-based variable thresholding technique produced much higher voxel count variation, ranging from -1.0% to 1.5%. This results from indistinct minima between the air and bone peaks, adding variability to the location of the thresholding boundary and introducing greater errors. In contrast, the air and bone peaks are sharp and consistent and hence easier to distinguish, and are subject to less variability. This allows a consistent definition of a threshold in the centre-based thresholding method.

The application of a local thresholding technique reduced the variability between scans to below 0.25%, however as for the static thresholding technique the largest variation still occurs over the first 90 minutes. This small change is likely due to the locally occurring gradient change in the pixel values and is therefore more difficult to account for.

Implications and future work

The recent improvement in X-ray CT acquisition times has resulted in an increasing number of time-lapse studies, providing an important new tool for understanding the behaviour of materials [4, 27, 28] using both laboratory- and synchrotron-based systems. Such studies place greater emphasis on understanding how materials change in-situ [28, 29] by tracking phenomena such as secondary phase formation, crack growth [30], or material deformation. A key element of any X-ray CT time-lapse experiment is therefore the ability to quantify any changes observed throughout the progress of the experiment. Here we demonstrate that changes in thresholded voxel counts occur due to the steady build-up of charge in the detector (Figures 2b & 3b), even when changes in the source have been minimised. The detectors in this study...
reached an equilibrium between charge production and dissipation after approximately 120 minutes, although we note that this is likely to vary between detector designs. On the basis of this work, we suggest detectors should be continually exposed to incident X-ray for a period prior to the start of an experiment when conducting time-lapse studies or the use of more advanced thresholding techniques that account for these changes. This is especially important in experiments where small changes through time need to be quantified with a high degree of accuracy.

Below we provide three examples where the ability to distinguish between subtle changes in materials volumes is particularly important.

Within the medical sciences, chronic bone disorders such as osteoporosis, osteopetrosis, osteoarthritis and Paget’s disease are often diagnosed on the basis of symptoms related to bone geometry as determined via medical-based X-ray and increasingly CT scans. Such disorders are often discernible only as subtle changes in bone volume or bone architectural properties such as trabecular thickness or anisotropy between study groups [17-19]. Typical clinical trials set up to investigate the efficacy of a particular treatment involve the use of model laboratory species such as rats and mice, as considered in the present study. In order to achieve statistical significance, trials may comprise a large number of murine osteological samples from various treatment groups, CT scanned over the course of one- to several days. The results outlined above urge caution when CT scanners are operational throughout the day in order to achieve high throughput of samples, as intrinsic instrument variation may mask the biological signal of interest between samples. Furthermore, time-lapse CT studies are increasingly being applied to the field of bone biomechanics to understand the strain behaviour of osteological samples subject to load [31]. The variability in grey scale histograms for a given sample across successive CT scans described here may impact considerably upon reconstructed volumes. Further research into the effect of instrument variability upon the digital volume correlation methodology and resulting strain maps is therefore required.

In nuclear materials studies, relatively small changes over a total voxel count may result in a significant difference when analysing microporous structures, such as that of graphite. The internal microstructure and porosity of graphite are increasingly studied using X-ray computed tomography [32-34] as a non-destructive method especially due to its developing capacities, including increased resolution and minimised time of scan. Graphite is one of the key components of nuclear reactor cores in the UK. Although graphite has been a subject of numerous studies since the fundamentals of nuclear graphite theory were established [35, 36], it has now become critical to understand the microstructural changes of graphite under irradiation, as the models initially developed to describe graphite behaviour with increased irradiation diverge from those observed at high doses. X-ray CT technique can be used to characterise the porous media by its porosity, pore size distribution and pore interconnectivity. These affect the mechanical properties of graphite as well as gas flow through its porous network. Gas is used as a cooling medium for
Advanced Gas-cooled Reactors (AGRs). When excited by gamma irradiation it causes radiolytic oxidation of nuclear graphite resulting in its pore structure change and bulk density decrease. This is one of the major concerns in reactor core integrity. Investigation of the microstructural changes of graphite along with porosity change is an important component in understanding the overall graphite behaviour under irradiation and oxidation conditions. The interpretation of the X-ray CT graphite porosity data is sensitive to the threshold method used: due to presence of fine interconnected pores within the network the variation in threshold value may either unite neighbouring pores or divide the whole pores into discrete structures. Although the change in total pore volume in graphite on macroscopic scale due to intrinsic variation of the X-ray CT data may be of a few percent this difference is large enough to produce a pronounced change in pore size distribution calculated using fixed (standard) grey scale value thresholding.

In geological applications the difficulties inherent in the analysis of complex natural systems – often with low attenuation contrast and subtle changes through time – may mask the impact of issues highlighted in this study. Nevertheless, time lapse CT studies are becoming increasingly common in some areas of the earth sciences. On such field is the examination of porous media for petroleum [37] and hydrology [38] applications – the uncertainties highlighted here could impact on quantification of changes pore volume, connectivity, pore-size distribution and pore geometry, in addition to analysis of rock fracture and heterogeneity development. The same issues will impact on the study of weathering of building stones [39] and soils [40]. Furthermore, recent reviews have highlighted that time-lapse CT could have a great impact on our understanding of root systems in soil [41] - where uncertainties will impact our understanding of both the nature of pores, but also conceivably root-growth, which can be followed with object tracking methods. Other areas where time lapse CT could have significant impact include monitoring structural dynamic processes, analyses of mineral processing and leaching, and fluid flow [42]. In all these areas, careful consideration of experimental procedure based on the findings presented herein will be required to minimise errors.

Conclusions

This study demonstrates that even when experimental procedures are employed to minimise changes in an X-ray source, variability resulting from the detector impacts on both scan histograms and voxel counts. This has particular relevance to temporal studies involving X-ray CT to characterise small changes. For applications such as those detailed in this study requiring an accelerating voltage up to 60kV the variability of the data due to the instrument can vary up to 1.5%. This assumes that the same threshold range is used for all datasets. This variation is caused by the build-up of charge on the detector system, which requires time (in the study 120 minutes) to stabilise.
The use of reference images at the start of a scan or regular intervals are usually advised for X-ray CT systems, as a method of correcting for changes (usually the source) in the instrument. In this study the use of new reference images did not result in effectively resetting the greyscale range of the outputted data. However results showed that the longer the detector is left to discharge the smaller the variability in data (0.3%).

The ability to account for changes in the X-ray instrument are very difficult to evaluate due to the inherent variability in pixel response associated with X-ray detectors, as well as potential fluctuations in X-ray sources themselves. The simplest method of minimising the instrument variation is to allow the detector to attain stabilisation – in this study this was after 120 minutes, however this approach is time consuming and still shows some variability. A far better approach to minimising the impact of instrument variation can be achieved by combining the above with the appropriate use of more robust thresholding techniques.

In simple two phase systems the selection of the thresholding range based upon the local minima in well-defined systems is a popular method. In this study it was shown to result in far greater variability (2.5%) which can be attributed to the difficulty of identifying the minima due to the increased noise. The application of a thresholding range based upon the central point method reduced variability to less than 0.4%. The latter approach significantly reduces the variability and is computationally simple to apply resulting is quick analysis times. This form of global thresholding is ideal for simple clearly defined two phase systems (Bone & air).

The application of a locally adaptive thresholding technique reduces the variation further to 0.25%, although this may still be a large number of voxels! For example, if the scan is 50% bone and you have a 2000*2000*2000 volume, the variation is 10 million voxels! These results show that locally adaptive thresholding techniques can minimise the detector variability in temporal X-ray CT studies to an acceptable level. In addition these techniques are a more robust method of segmentation and do not have the limitations that global thresholding techniques can display over large volumes. In more complex multiphase systems where local adaptive thresholding techniques are not appropriate, the use of marker based thresholding techniques such as watershed methods may give comparable results however this requires further investigation.

The results presented in this study show the variability that occurs during an X-ray CT scan series on the Nikon X-TEK 225kV instruments in the energy range of 40 to 50kV. This variability will be present in all types of X-ray CT instruments due to the X-ray source and detectors designs, and will occur over the entire energy range. Further studies need to be conducted in order to ascertain the impact of the X-ray energy range upon the detector response and the degree of variation in other detector systems.
References


