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

2 T. Lowe^{1*}, C. Simpson², D. Jones³, R. Garwood⁴, C.A. Brassey⁵, Z. Mileeva⁶, A. Ramsey⁷ R.S. Bradley¹, R.J.

3 Cernik¹, P.J. Withers^{1,2}.

4 1. Manchester X-ray Imaging Facility, School of Materials, The University of Manchester, M13 9PL UK.

5 2. The Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot, Oxfordshire OX11 0FA, UK.

6 3. Waveform Computing Ltd, Manchester, M20 2XU, UK

7 4. School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Manchester,

8 M13 9PL, UK.

9 5. School of Life Sciences, The University of Manchester, Manchester, M13 9PL, UK.

10 6. School of Mechanical, Aerospace & Civil Engineering, The University of Manchester, M13 9PL, UK

11 7. Nikon X-Tek Systems Ltd, Tring Business Centre, Icknield Way, Herts, HP23 4JX, UK

12 **Abstract**

13 High resolution laboratory X-ray Computed Tomography (CT) systems are capable of providing three

14 dimensional (3D) images at spatial resolutions ranging from hundreds of microns to better than 100nm.

15 Increasingly such images are being used to quantify features (porosity, defects, geometry, phase fractions,

16 etc) by segmenting the images into different regions on the basis of greyscale values. As a non-destructive

17 technique X-ray CT is particularly well suited to time-lapse (3D+time) studies, however, temporal variability

18 in both the source and or detector can alter the grey scale values of the reconstructed volumes. In this

19 paper we examine the extent of the greyscale variation as a function of time. Our results show that

20 detector performance is not constant over time and that decreasing source accelerating voltage and

21 decreasing exposure times introduce greater variability. We examine the implications of temporal

22 variations in contrast on the quantitative analysis of 3D images. Our work demonstrates the dangers of

23 using global greyscale automatic thresholding and shows that a careful choice of thresholding technique,

24 coupled with pre-experiment conditioning of detectors can minimise variability in quantities obtained at

25 different times.

27 Introduction

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

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

46 Rather than use attenuation contrast (grey level) as a direct indicator of density the majority of studies now
47 use changes in grey level to distinguish specific regions. In this approach a feature is distinguished and
48 isolated by applying a threshold range based upon its greyscale values, allowing subsequent visualisation
49 and characterisation of the feature in 3D. This allocation of pixels to phases or features according to grey
50 level ranges, known as segmentation, has markedly reduced the use of calibration standards because
51 absolute levels are not required to distinguish regions of interest. While this is in many cases sufficiently
52 accurate it should be noted that the grey level histogram is usually composed of overlapping peaks.
53 Consequently small changes in the thresholding range (selected either manually [6, 7] or automatically [8-
54 12]) can result in significant variations in the volume and morphologies of the identified phases [4]. The
55 extent to which such changes will affect the quantification is a function of the thresholding method chosen.
56 At present there are no agreed standards regarding either the collection of X-ray CT data or data
57 processing, although the ASTM has issued guides regarding both [13, 14]. The use of a specified threshold
58 range over an entire data volume is known as global thresholding. Global thresholding is the easiest
59 method of segmentation implementation and as such is the most popular method amongst CT researchers.
60 Unfortunately CT images can display a number of artefacts such as a beam hardening [15, 16]. Because they
61 affect the grey levels such artefacts can seriously affect the segmentation. Those grey level artefacts which

62 are long-range, such as beam hardening, can be partially accounted for using locally adaptive segmentation
63 methods. Adaptive thresholding techniques segment features based upon the local grey scale change over
64 a pre-defined local area, this approach takes into account any background changes that occur over larger
65 volumes resulting in a more accurate feature segmentation and a review of thresholding techniques has
66 been considered by others such as [10].

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

75 Changes in the source are usually considered to be negligible over time, however, there are a number of
76 reasons why the source might vary over time. In the laboratory these could arise from thermal expansion
77 induced movement of the target or variations in the electron beam that strikes it, or voltage instabilities
78 (<1% for modern sources) or target degradation. For synchrotron sources this might arise from beam
79 instabilities or drifts.

80 Numerous factors can cause a variation in the response of X-ray detectors over time. These will directly
81 affect a detector's maximum frame rate, energy and contrast sensitivity, and signal to noise ratio. Hence,
82 temporal variation should be considered for specific detector systems, however, some issues will affect all
83 detectors as they age. The most common problem is damage accumulated over months to years, manifest
84 in the form of dead (non-responsive) pixels or defective ones whose behaviour differs from their
85 neighbours. Most systems use calibrations to correct the resulting artefacts. Another practical problem
86 encountered in CT is that of residual (ghost) images following exposure, caused by the time required for
87 pixels to dissipate residual charge. This can cause significant artefacts in scans where the detector has been
88 over exposed, but will dissipate with time, and can be easily avoided by adjusting scan settings in line with
89 manufacturer's guidelines. Another problem that impacts some detectors is a gradual accumulation of
90 charge, directly altering the sensitivity. This can be avoided by discharging the detector at the end of each
91 scan, effectively returning the system to a zero reference point. Finally, operating temperature can affect a
92 detector's performance; however modern detectors have a high degree of temperature stability and are
93 usually housed within temperature controlled units.

94 This study investigates the instrumental and analytical issues that hamper the accuracy, reliability and
95 robust quantification of objects overtime and aims to identify methods to minimise these effects on image
96 quantification of a time lapse series. The two main causes of instrument variation are:

- 97 I. The X-ray detector - X-ray detectors are optimised for a specific energy range that is determined by its
98 constituent energy sensitive materials. This results in detectors demonstrating a variability in response
99 time related to the incident photon energy spectra produced by the X-ray source. In addition due to the
100 charge produced by the incident photons, X-ray detectors are susceptible to charging over time which
101 can directly affect the performance. In this study the detector exposure time during X-ray CT batch
102 scans is investigated for three different energy settings.
- 103 II. The X-ray source – Changes in the emitted X-ray spectra will directly affect the X-ray detector response
104 and consequently the grey scale values in the reconstructed X-ray CT scan. Changes in the X-ray source
105 can be due to:
- 106 a. Instability of the X-ray tube accelerating voltage
 - 107 b. Changes in the X-ray spot on the target material. This can be caused by heating of the target
108 material over time due to poor cooling or degradation of target material due to damage
 - 109 c. Degradation of the X-ray filament over time results in a decreased photon count. This usually
110 results in the application of a different accelerating voltage in order to achieve the appropriate
111 transmission and or flux through the specimen, however this results in a change in the emitted
112 X-ray spectra

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

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

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

126

127 Experimental

128 Scanning Experiments

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

137 The specimen was analysed using a Nikon 225/320 kV CT system housed within a customised bay with a 2k
138 x 2k Perkin Elmer 1621-16-bit amorphous silicon flat-panel detector having 200 micron pixel pitch and an
139 aluminium window (XRD 1621 AN3 CT grade), a 225kV X-ray source with a multi-metal reflection target (in
140 all cases a tungsten target produced the best signal to noise ratio for the accelerating voltage of 40-60kV).
141 2001 projections were acquired over 360° with the specimen to X-ray source and specimen to detector
142 distances of 34.2mm and 1007.0 mm respectively, resulting in a reconstructed voxel size of 6.8µm. To avoid
143 over-exposing the detector, the current was adjusted to obtain an average photon counting rate of
144 approximately 55000 counts per pixel per projection. For all scans a single reference/calibration image was
145 used for each scan series. In addition, the focus was optimized for the low energy spectrum and the system
146 alignment was rechecked in order to optimize resolution.

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

149 24hrs prior to the experiment the instruments was set with the specimen and focused at 50kV. The X-ray
150 source (under vacuum) and detector were turned off for 24hrs to ensure no previous experiments could
151 influence the results. Before each scan series:

- 152 • The X-ray source was conditioned at 225kV to ensure beam stability (± 1 kV).
- 153 • After beam conditioning the X-ray source was turned on and a lead shield placed in front of the X-
154 ray source to avoid any incident photons on the detector. This precaution was taken to ensure that
155 the X-ray spot on the target achieved an equilibrium size and shape. However it is noteworthy that
156 experiments conducted without this step did not affect the results.
- 157 • A reference image was then collected and the scan series (Table 1) was then started

158 At the end of each scan series the instrument source was turned off to minimise filament degradation and
159 the instrument was left for 7 hours before starting the above three steps for the next scan series (Table 1).

160

Series Run No	Voltage /kV	Current / μ A	Projection time / ms	Total number of scans
I.1	50	80	2	8
I.2	50	170	1	12
I.3	50	380	0.5	15

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

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

167

Series Run No.	Voltage /kV	Current / μ A	Projection time / s	Total number of scans
II.1	40	380	1	10
II.2	50	170	1	10
II.3	60	105	1	10

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

171

172 Experiment III: To determine the extent to which detector charging may contribute to variability in image
173 acquisition over time, and whether the application of new reference images may correct for this effect. For
174 this experiment the specimen was scanned at 50kV, 170 μ A and a projection time of 1 second. A reference
175 image was taken at the start of the series was used for the first four scans that were separated by several
176 hours (during this time the X-ray source was turned off) to ascertain the effect of detector charging upon

177 image variability. Further scans were then collected using new reference images to determine whether the
178 application of new reference images resulted in the reduction of image variability as is sometimes
179 recommended by instrument manufactures.

180 Image segmentation methods

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

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

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

¹ See supplementary information

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

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

218 Trial 3: Global thresholding based on mid-points between peaks. This method is illustrated in Figure 1a and
219 the segmentation results are shown in Figure 1d. For this method a simple thresholding function using the
220 SciPy signal processing package function in Python was used to locate the peaks in the intensity spectrum
221 (the raw code can be found in the supplementary information). The midpoint was the calculated based
222 upon these positions as shown in Figure 1a. This method is popular when the minima between peaks
223 (method 1 above) are not well defined, which can cause repeatability issues relating to image noise. In
224 cases where peaks are well defined this method can result in reduced variability in the thresholding. As for
225 Method 1 the boundary conditions were found for the first scan and then applied to all other scans in the
226 series run as a reference point. This thresholding technique is referred to as “static centre”. In this
227 investigation this method produced a better segmentation of the fine trabecular structure (Region 2 Figure
228 1d) at the expense of over-estimating the main bone section (Region 1 Figure 1d) where many of the pores
229 are no longer present when compared to the unsegmented original data in Figure 1b.). A direct
230 comparison of the number of pixels selected by the centre method verses the minima thresholding
231 technique shows an increase of 3.35%.

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

236 Trial 5: As summarised in [4] locally adaptive thresholding technique have certain advantages over global
237 thresholding techniques. While the global thresholding techniques (methods 1-4 above) does not take into
238 account the change in grey scale values between neighbouring voxels, the local thresholding techniques
239 look for changes in gradient between neighbouring voxels over a specified range. By taking into account
240 local changes in the greyscale values locally adaptive thresholding techniques are thus less prone to
241 changes in absolute greyscale across the volume as might occur due to X-ray CT artefacts such as beam

242 hardening or the cone beam effect for example. The latter is shown in Figure 1e where this method shows
243 a better segmentation of the fine trabecular structure (Region 2 Figure 1e) while still discriminating the
244 pores in the main bone (Region 1 Figure 1d), although the latter are possible slightly over estimated when
245 compared directly with the original segmented image (Figure 1b). A direct comparison of the number of
246 pixels selected by the adaptive method verses the minima thresholding technique shows an increase of
247 4.05%. In this study the SciPy signal processing package function in Python was used to perform the
248 thresholding. In this function a Gaussian fit was applied to the data and a kernel (or window) size of 60
249 pixels was applied.

250 Results and discussion

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

252 Figure 2a shows the temporal variation in grey scale distribution recorded for successive scans based on 1s
253 projection acquisition times. A shift in the bone peak position to a lower grey scale value is evident with
254 time being most marked for the first few scans. Similar trends occur for projection times of 0.5 and 2.0
255 seconds (Table1). Changes in the magnitude and position of the bone peak directly impact upon the
256 thresholded voxel count (Figure 2b), resulting in 0.6-1.5% variation in the estimated bone volume between
257 scans settling down after 90mins or so.

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

264 Experiment II: Variability as a function of accelerating voltage

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

273 the quantitative problems that are introduced when the accelerating voltage is increased during in-situ
274 studies.

275 It is clear from Figure 3b) that the grey scale variations over time lead to changes in the segmentation and
276 that this appears to plateau more quickly at lower voltages (~30mins at 40kV) compared to higher voltage
277 (~100mins at 60kV). This can be explained as follows; when incident X-ray photons first interact with the
278 detector (more specifically when the signal from the scintillator hits the amorphous Si detector array),
279 electron-hole pairs are formed. The number of these produced is directly proportional to the incident
280 photon energy and the number of photons. Electron-hole pairs then travel to the anode and cathode on
281 the detector array where their presence is registered. The greyscale value from each pixel - which is
282 proportional to the registered electrons on the cathode - is then recorded but charge dissipation of the
283 detector is not 100% efficient over the acquisition time. The observed shifts appear to result from a gradual
284 build-up of charge in the detector which ultimately approaches a dynamic equilibrium where the charge
285 generated by the incident photons is equal to the charge dissipated at the cathode. The increased time
286 required for the segmentation volumes to plateau with increased accelerating voltage is likely linked to
287 the associated increase in charge production within the detector. The above effect could be associated with
288 heating of the detector over time, however this can be discounted due the X-ray source and detector unit
289 being setup in a large walk-in chamber that is temperature controlled. In addition the actual energy that is
290 incident upon the detector is below 0.5W of power and will have a negligible effect.

291 Experiment III: Detector charging and application of reference images

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

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

306

307 Impact of segmentation techniques

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

317 To assess the impact of this experimental choice, Figure 5 illustrates the variation in voxel counts for the
318 thresholding techniques described previously. The centre-based variable thresholding methodology
319 provided a voxel count variation of $< 0.4\%$ between different scans within the same batch. This compares
320 favourably with the static thresholding method, which has a maximum error of 1.4% . However, the minima-
321 based variable thresholding technique produced much higher voxel count variation, ranging from -1.0% to
322 1.5% . This results from indistinct minima between the air and bone peaks, adding variability to the location
323 of the thresholding boundary and introducing greater errors. In contrast, the air and bone peaks are sharp
324 and consistent and hence easier to distinguish, and are subject to less variability. This allows a consistent
325 definition of a threshold in the centre-based thresholding method.

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

330 Implications and future work

331 The recent improvement in X-ray CT acquisition times has resulted in an increasing number of time-lapse
332 studies, providing an important new tool for understanding the behaviour of materials [4, 27, 28] using
333 both laboratory- and synchrotron-based systems. Such studies place greater emphasis on understanding
334 how materials change in-situ [28, 29] by tracking phenomena such as secondary phase formation, crack
335 growth [30], or material deformation. A key element of any X-ray CT time-lapse experiment is therefore the
336 ability to quantify any changes observed throughout the progress of the experiment. Here we demonstrate
337 that changes in thresholded voxel counts occur due to the steady build-up of charge in the detector
338 (Figures 2b & 3b), even when changes in the source have been minimised. The detectors in this study

339 reached an equilibrium between charge production and dissipation after approximately 120 minutes,
340 although we note that this is likely to vary between detector designs. On the basis of this work, we suggest
341 detectors should be continually exposed to incident X-ray for a period prior to the start of an experiment
342 when conducting time-lapse studies or the use of more advanced thresholding techniques that account for
343 these changes. This is especially important in experiments where small changes through time need to be
344 quantified with a high degree of accuracy.

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

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

362 In nuclear materials studies, relatively small changes over a total voxel count may result in a significant
363 difference when analysing microporous structures, such as that of graphite. The internal microstructure
364 and porosity of graphite are increasingly studied using X-ray computed tomography [32-34] as a non-
365 destructive method especially due to its developing capacities, including increased resolution and
366 minimised time of scan. Graphite is one of the key components of nuclear reactor cores in the UK. Although
367 graphite has been a subject of numerous studies since the fundamentals of nuclear graphite theory were
368 established [35, 36], it has now become critical to understand the microstructural changes of graphite
369 under irradiation, as the models initially developed to describe graphite behaviour with increased
370 irradiation diverge from those observed at high doses. X-ray CT technique can be used to characterise the
371 porous media by its porosity, pore size distribution and pore interconnectivity. These affect the mechanical
372 properties of graphite as well as gas flow through its porous network. Gas is used as a cooling medium for

373 Advanced Gas-cooled Reactors (AGRs). When excited by gamma irradiation it causes radiolytic oxidation of
374 nuclear graphite resulting in its pore structure change and bulk density decrease. This is one of the major
375 concerns in reactor core integrity. Investigation of the microstructural changes of graphite along with
376 porosity change is an important component in understanding the overall graphite behaviour under
377 irradiation and oxidation conditions. The interpretation of the X-ray CT graphite porosity data is sensitive to
378 the threshold method used: due to presence of fine interconnected pores within the network the variation
379 in threshold value may either unite neighbouring pores or divide the whole pores into discrete structures.
380 Although the change in total pore volume in graphite on macroscopic scale due to intrinsic variation of the
381 X-ray CT data may be of a few percent this difference is large enough to produce a pronounced change in
382 pore size distribution calculated using fixed (standard) grey scale value thresholding.

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

397 Conclusions

398 This study demonstrates that even when experimental procedures are employed to minimise changes in an
399 X-ray source, variability resulting from the detector impacts on both scan histograms and voxel counts. This
400 has particular relevance to temporal studies involving X-ray CT to characterise small changes. For
401 applications such as those detailed in this study requiring an accelerating voltage up to 60kV the variability
402 of the data due to the instrument can vary up to 1.5%. This assumes that the same threshold range is used
403 for all datasets. This variation is caused by the build-up of charge on the detector system, which requires
404 time (in the study 120 minutes) to stabilise.

405 The use of reference images at the start of a scan or regular intervals are usually advised for X-ray CT
406 systems, as a method of correcting for changes (usually the source) in the instrument. In this study the use
407 of new reference images did not result in effectively resetting the greyscale range of the outputted data.
408 However results showed that the longer the detector is left to discharge the smaller the variability in data
409 (0.3%)

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

416 In simple two phase systems the selection of the thresholding range based upon the local minima in well-
417 defined systems is a popular method. In this study it was shown to result in far greater variability (2.5%)
418 which can be attributed to the difficulty of identifying the minima due to the increased noise. The
419 application of a thresholding range based upon the central point method reduced variability to less than
420 0.4%. The latter approach significantly reduces the variability and is computationally simple to apply
421 resulting in quick analysis times. This form of global thresholding is ideal for simple clearly defined two
422 phase systems (Bone & air).

423 The application of a locally adaptive thresholding technique reduces the variation further to 0.25%,
424 although this may still be a large number of voxels! For example, if the scan is 50% bone and you have a
425 2000*2000*2000 volume, the variation is 10 million voxels! These results show that locally adaptive
426 thresholding techniques can minimise the detector variability in temporal X-ray CT studies to an acceptable
427 level. In addition these techniques are a more robust method of segmentation and do not have the
428 limitations that global thresholding techniques can display over large volumes. In more complex multiphase
429 systems where local adaptive thresholding techniques are not appropriate, the use of marker based
430 thresholding techniques such as watershed methods may give comparable results however this requires
431 further investigation.

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

437

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