DOCTOR OF PHILOSOPHY

# A qualitative and quantitative analysis of the developing human lumbar vertebral column

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## Appendix 6.4: µCT Scanning Methodology

After loading the Inspect X software, the scan was named at the ‘Start’ screen. Scans were named relating to their Scheuer collection specimen number and the level of the lumbar vertebral column from which they arose, for example SC-065 L1. The second stage of scan set-up was the ‘Position and Optimise’ screen (Figure 1). A second screen showing a live x-ray image was used to assess manual changes made to the scan conditions. A number of parameters could be controlled manually. The first set of parameters related to the position of the bone within the cabinet (Figure 1, red circle). The X and tilt axes remained locked at all times to prevent any movement in these directions which could have led to the vertebra moving diagonally or falling from the metal table. Directional joysticks were used to move the vertebra into the centre of the screen. It was important for the vertebra to be centred as this minimised the space occupied by the vertebra during the scan and kept the FOV as low as possible. Once centred, the joysticks were then used to zoom in or out so that the entire vertebra was within the FOV. The sample was also rotated 360° to confirm that the bone was contained in its entirety within the FOV. It was easy to identify cases where vertebrae sat off centre at this point, as the entire bone would leave the FOV at the right and/or left sides of the screen. In these cases, the vertebra was readjusted as described above. The effective pixel size (µm) was related to the FOV, with increased zoom leading to smaller effective pixel size, and decreased zoom leading to a larger value. The values in this section were all individual dependent and based upon vertebra size. Larger vertebrae required larger FOVs which led to a larger effective pixel size and hence a lower resolution.

The second section of the ‘Position and Optimise’ screen involved the setting of the beam energy (kV), beam current (uA) and machine power (W), which were also individual dependent (Figure 1, blue circle). The scan conditions for each individual can be seen in Appendix 6.3. During the setting of these values, it was important that the machine power did not exceed the effective pixel size. The values of these parameters were based on the live x-ray image produced, to achieve a clear image with distinct differentiation between bone tissue and background. While this was achieved by eye, the minimum and maximum grey levels were also used as an indicator (Figure 1, green circle). Firstly, it was important that the maximum grey level (a value of 63,353) did not become saturated. Secondly, optimal differentiation occurred when the maximum grey value was around 3-4 times that of the minimum grey level. These values could be seen on the right-hand side of the screen and were updated instantly as any changes were made to the parameters of the scan. For example, if the chosen parameters of the scan gave a minimum grey level value of 10,000, the optimal value for the maximum grey level would be around 40,000. In some cases, particularly for smaller vertebrae with the smallest effective pixel sizes, this differentiation in grey levels was more difficult to achieve. In these cases, the optimum differentiation possible was chosen without saturation occurring (Figure 6.2 is an example of this). For all individuals, the exposure (fps, ms) and gain (X dB) were kept the same, at 2.00fps, 500ms and 24X dB respectively (Figure 1, yellow circle).

Once the ‘Position and Optimise’ screen had been completed, the ‘Correction’ screen created a shading correction of the background and applied this to the scan. Once applied, it was important to check whether the addition of the shading correction had caused the scan to become saturated. This was accomplished by identifying whether the minimum grey level had reached a numerical value of 65,353. In cases where this had occurred, the beam energy of the scan was decreased, and a new shading correction created and applied. The next two tabs, ‘Reconstruction’ and ‘Volume Analysis’ were not used, as separate software was available for this purpose.

At the ‘Acquire Dataset’ screen, a number of scan conditions were available based on the number of projections and the number of frames per projection for the scan. The number of projections is the number of smaller rotations made by the metal table within the cabinet, to total 360°. An increased number of projections would lead to smaller degree increments and therefore more rotations to reach 360°. The number of frames per projection is the number of x-ray images taken at each projection, an average of these images gives the final image for that projection. A preliminary study was completed to identify the optimal scan parameters for the sample, which can be viewed in Appendix 6.1. Individuals were scanned at 1,682 projections with 2 frames per projection. The duration of each scan was 28 minutes and 26 seconds.

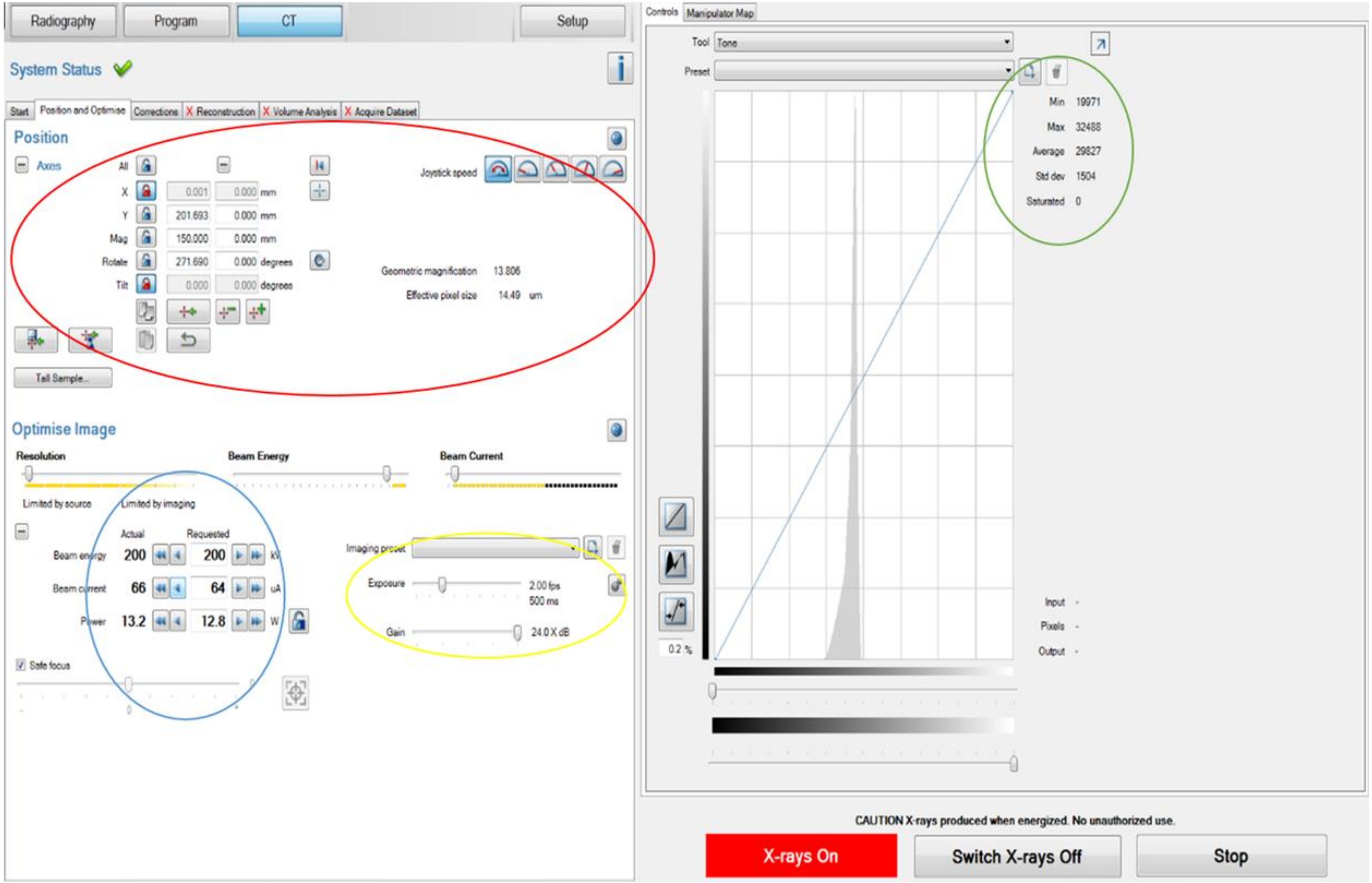


*Figure 1: The Position and Optimise screen of the Inspect X software.*