

Evaluation of Three-dimensional Image Registration Methodologies for *In Vivo* Micro-computed Tomography

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Abstract—The advent of *in vivo* micro-computed tomography (micro-CT) provides a novel approach to measure the temporal adaptation of bone micro-architecture within an individual. Spatial alignment in the scanner between serial scans is challenging, but three-dimensional image registration can be used to superimpose the resulting image data, thus ensuring consistent regions of interest (ROI) for analysis. There have been several approaches to image registration developed, yet little is known about their application to high resolution micro-CT data. The purpose of this study was to explore combinations of three image registration similarity measures and three image interpolators, in addition to multi-resolution registration configurations, for assessment of computational efficiency and accuracy on both *in vitro* and *in vivo* micro-CT data. Accuracy measures were assessed by comparison with a gold-standard reference transform based on attached fiducial markers. It was concluded that a mutual information registration similarity measure with a linear image interpolator, applied at steps of increasing image resolution, provided the best compromise between accurate and efficient results. *In vivo* registration of tibial bone microstructure measured in an ovariectomized rat model provided consistent ROI thus demonstrating the usefulness of three-dimensional image registration for *in vivo* experimental and clinical micro-CT research. It is a technique that is poised to become commonly utilized for analysis of micro-CT data to diagnose and monitor efficacy of therapy in bone diseases.

Keywords—Image registration, Micro-computed tomography, Osteoporosis, Rat models, Tibial bone micro-structure.

INTRODUCTION

The measurement of bone micro-architecture provides novel insight into morphological and structural

adaptations of bone in diseases and joint injuries. Micro-computed tomography (micro-CT) is a key technological development in the past decade that provides fast, non-destructive assessment of bone architecture,³³ and has been instrumental for augmenting our understanding of disease progression (i.e., osteoporosis) and the efficacy of therapies to arrest or slow bone loss.

Micro-computed tomography was originally developed for *in vitro* measurements, therefore, typical study designs have been based either on bone biopsies from human patients or, in experimental animal models of human diseases, on cohorts of animals sacrificed at pre-defined time intervals to assess longitudinal changes during disease progression. The *in vitro* measurements provide “snapshots” in time of the three-dimensional (3D) bone micro-architecture which are important for studying bone; however, a limitation of *in vitro* study designs is that variability within groups can weaken the statistical power to detect important changes between time intervals – i.e., intra-group variability may obscure inter-group changes. Therefore, an improved study design would monitor bone adaptation within an individual so that group-wise averaging is not necessary. This would provide powerful insight into the processes of bone adaptation on an individual basis, and have the advantage of reducing the number of subjects (either human or animal) needed for research. The recent introduction of *in vivo* micro-CT for both human^{2,18} and animal^{7,39} research provides a new opportunity to monitor 3D micro-architectural changes on a temporal basis in an individual.

Accompanying the new *in vivo* technology are the challenges and opportunities to maximize the information that can be obtained from these temporal data. Although there are many possibilities to develop new and innovative approaches for analyzing these

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data,^{4,5,19,39} the obvious first step is to apply the traditional morphological tools established for *in vitro* micro-CT measurements^{12,13,28,37} to each micro-CT scan within an individual time series. However, repositioning of the subject in the scanner is problematic, and it is therefore, difficult to directly compare spatial regions of interest (ROI) from different time points. To maximize the effectiveness of *in vivo* micro-CT, it will be critical to use computer methods to superimpose 3D micro-CT data from different time points. Thus, consistent ROIs for morphological analyses can be ensured, and importantly, individual components of bone micro-architecture can be monitored longitudinally providing a novel opportunity to learn about bone adaptation.

The method of 3D data superposition, or image registration, involves generally a computer algorithm that iteratively searches for a combination of 3D position and orientation that best fits one image to another according to an optimization criterion based on a similarity measure. Registration of two different imaging modalities such as MR and CT data from the same patient is called *inter-modal* image registration for which the mutual information measure^{24,38} was specifically designed. Registration of *in vivo* micro-CT in time series involves only the one image modality, thus *intra-modal* registration similarity measures may be more suitable, such as normalized correlation or mean squares intensity difference.^{11,14} Accompanying the choice of a registration similarity measure is the choice of a method to *interpolate* the 3D data into different spatial positions. Interpolators have important implications on the registration accuracy and efficiency, and three commonly used interpolators, in the order of increasing complexity, include nearest neighbor, linear and *b*-spline interpolation.^{22,29} There are many possible combinations of registration measures and interpolators, and none of these techniques were specifically designed for micro-CT data where the high periodicity of the trabecular micro-structure¹⁷ could result in misregistration. Furthermore, large data sets (i.e., >1GB data) will require efficient registration techniques, such as multi-resolution registration where pre-registration steps are performed at artificially reduced image resolutions to improve solution efficiency.^{25,32,36} Serial measurements have been performed with MR data,¹⁶ and recently based on micro-CT data³⁹ that used the mutual information measure. It is clear that *in vivo* micro-CT studies will become more common as the technology becomes more widely available, therefore, it is important to establish which registration approaches are suitable for these unique datasets where detailed micro-structure is available.

Assessment of registration methods can be based on efficiency and accuracy. The former is important so

that high-throughput *in vivo* micro-CT systems can be effectively utilized. The latter is difficult to quantify because of a lack of reference gold-standard measures in adapting bone. One approach is to use histomorphological analysis of bone labels to compare with registration results,³⁹ however, in research involving systematic bone loss (i.e., disuse atrophy, osteoporosis) the labeling technique is limited. An established approach in the field of registration research is to use target registration errors (TRE) to measure alignment errors between two registered images.^{8,9,15} The TRE can quantitatively establish the relative accuracy of different combinations of image registration approaches to aid in the decision of an appropriate approach for micro-CT.

The mutual information similarity measure is a well-established method that is versatile for both intra- and inter-modal registration. Thus, it was hypothesized that the mutual information measure in combination with a simple linear interpolator could provide accurate and efficient 3D image registration for high-resolution micro-CT data. The purpose of this study was to evaluate possible combinations of established rigid registration approaches for superimposing 3D images of bone microarchitecture from micro-CT data to determine their relative accuracy and efficiency. Specifically, three registration similarity measures and three interpolation methods, and combinations of these approaches including multi-resolution registration, were tested. The registration approaches were evaluated for efficiency and accuracy on *in vitro* data sets against a ground truth solution obtained using fiducial markers, and subsequently the technique was demonstrated on *in vivo* micro-CT data.

METHODS

The registration procedures were tested for relative accuracy and efficiency using *in vitro* micro-CT first, followed by application of the methods to *in vivo* micro-CT data of an ovariectomized (OVX) rat model. The OVX rat model is commonly used for research into diseases such as osteoporosis.^{20,21,41}

Registration Measures and Interpolators

The approach to 3D image registration involves designating one image as fixed, *f*, and the other as moving, *g*, with the goal being to find an appropriate transformation that can be applied to the *moving* image to superimpose it onto the *fixed* image. The transformation determined by this procedure defines a rigid registration with six degrees of freedom. Our implementation of the 3D image registration approach

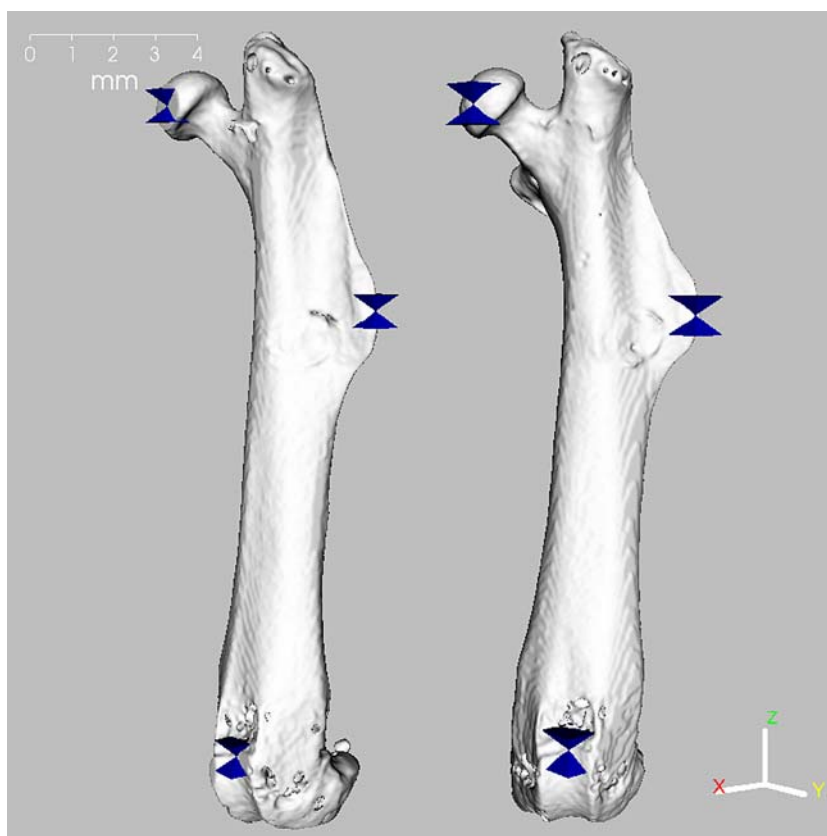


FIGURE 1. A minimum of three similar landmarks are selected interactively using software for 3D visualizations of the image data. These landmarks are used to calculate a transformation matrix, which is provided as an initial guess to the registration process.

involves two steps. First, the user provides a starting estimate of the transformation by selecting a minimum of three arbitrary landmarks and defining their corresponding positions in each 3D image. There is no requirement that these landmarks be of anatomic importance, only that they are identifiable in each image. To aid the identification of landmarks, software was developed to visualize the 3D data using a standard Marching Cubes algorithm²³ so that the user could interact with the data and select appropriate landmarks (Fig. 1). Secondly, the fully automated iterative process of image registration searches for a transformation that optimally superimposes the moving image onto the fixed image (Fig. 2). Three registration measures and three image interpolators were evaluated for the registration of the *in vitro* micro-CT data. For all combinations, a gradient descent optimizer was applied that moved in a step-wise fashion in the direction of the local derivatives. The gradient was recomputed after each step, such that a new direction in parameter space was considered each time (steepest gradient approach). Although the ideal step size results in a minimum number of iterations while keeping the optimization stable, selection of an appropriate step size is challenging. The more conservative approach is

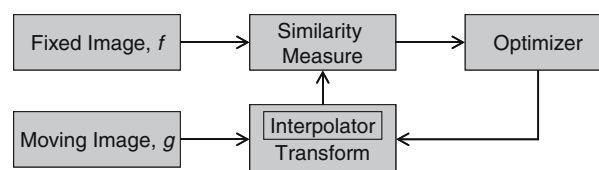


FIGURE 2. The registration process flow diagram. Two 3D images are designated as fixed and moving images, respectively. A transformation is applied to the moving image and the image data is interpolated into a new coordinate system such that the registration similarity measure can evaluate the degree of superposition. Based on the output of the similarity measure, a gradient descent optimizer adjusts the transformation. The iterative process continues until a solution has been obtained based on a constraint on the number of iterations or a convergence criterion.

to use a small step size, but this may result in a long solution time. Therefore, we adjusted the step size dynamically throughout the optimization. The step size was reduced when the step taken resulted in an updated position in parameter space away from the optimum (i.e., increased function value), and was increased otherwise (to speed up convergence).

The three registration measures tested included mean squared difference of intensities (MS), normalized correlation (NC) and mutual information (MI).⁴²

The first two measures compare image intensities for each voxel (i.e., 3D pixel), and therefore, are suitable only for intra-modal registration (i.e., CT to CT).¹⁴ The calculation of the MS and NC measures are based, respectively, on the following minimizations:

$$\text{MS}(f, g) = \frac{1}{N} \sum_i^N (f_i - g_i)^2, \quad (1)$$

and

$$\text{NC}(f, g) = - \frac{\sum_i^N (f_i \cdot g_i)}{\sqrt{\sum_i^N f_i^2 \cdot \sum_i^N g_i^2}}, \quad (2)$$

where f is the fixed image, g is the moving image, N is the number of voxels, and f_i or g_i are the intensities of i^{th} voxels in each image at spatially corresponding positions after applying the rigid body transformation. The third measure, MI, is based on the minimization of joint entropy between two images.^{24,38,40} Based on a probability density function (PDF), the marginal entropies $H(f)$ and $H(g)$ for the fixed and moving images, and the joint entropy $H(f, g)$, are calculated such that for a given transformation the joint entropy is minimized.^{24,40}

$$\text{MI}(f, g) = H(f) + H(g) - H(f, g), \quad (3)$$

thus, the optimization determines the position and orientation such that mutual information is maximized. We report the negative mutual information measure so that the optimizer determines a minimum value to be consistent with the other similarity measures. The MI measure was designed for inter-modal registration (e.g., MR to CT data) as the two images do not need to have similar voxel intensities over the image space. A particular form of mutual information, named Mattes mutual information, was utilized^{26,27} where the PDFs are estimated using Parzen histograms. The number of spatial samples was set to 20% of the total number of voxels, and the joint histogram was calculated based on 50 bins for each image (i.e., a 50×50 joint histogram). The image derivatives used by the optimizer were computed using the central difference method.

The three 3D image interpolators that were tested, in order of increasing computational complexity, were nearest neighbor (i.e., select intensity of nearest grid position), linear (i.e., linear interpolation of grid intensities) and cubic b -spline (i.e., higher order interpolation).³⁰ These three interpolators are standard methods used in many scientific fields.

The registration measures were tested while keeping the image interpolator constant (linear interpolator), and the interpolators were tested with a constant similarity measure (normalized correlation). For each

combination, multi-resolution registration was applied to test the effect of artificially reducing image resolution where isotropic downsizing was performed by voxel averaging to improve registration efficiency. The convergence of the registration measures and interpolators, and the time per iteration for each downsizing factor tested was recorded for all tests.

In Vitro Image Registration

The test data comprised of repeated micro-CT scans of a C57/B6 adult mouse femur. The study was approved by The University of Calgary Animal Care Committee. Six glass beads (approximately 1 mm diameter) were fixed to the femur (super glue) to provide reference marks (Fig. 3). The sample was scanned (SkyScan 1072, SkyScan, Belgium) five times providing a nominal isotropic resolution of 32 μm , and the femur was removed and replaced between scans to deliberately cause repositioning error. Subsequent to scanning, the glass beads were artificially removed from the images (i.e., reset to zero attenuation value in the 3D images) by applying a custom-developed masking algorithm that was based on the relatively high attenuation values of the glass beads relative to the bone tissue (a factor of three higher attenuation; Fig. 3). Although the beads were distributed over the entire femur and were used as the gold standard for registration, only the distal 6 mm were subsequently used for the automated registration. The distal 6 mm corresponded to an image containing approximately 6.86×10^6 voxels (190^3). The distal region of interest used for automated registration is representative of future applications of the methodology where cancellous structures are of interest. The micro-CT datasets were registered without prior image processing (i.e., filtering, segmentation), and the advantage of using the *in vitro* data is that the confounding effects of bone growth or adaptation found *in vivo* were excluded.

The five images provided 10 possible image pairs to be registered. For each set, a starting estimate of the transformation was determined by picking landmarks, and these landmarks were held constant for all subsequent registration tests. All registrations were initially performed with a downsizing factor of 3.5 (300 iterations) followed by a downsizing factor of 1.0 (full resolution; 100 iterations). For each pair of registered images, the three variables tested were the registration measure, interpolator, and downsizing factor. The registrations were performed on a standard workstation (Sun Blade 2500, UltraSPARC IIIi processor, 1.28 GHz, 2 GB RAM, Sun Microsystems, CA). The output of each registration was the transformation matrix, T , that optimally superimposed the two images according to the applied registration measure.

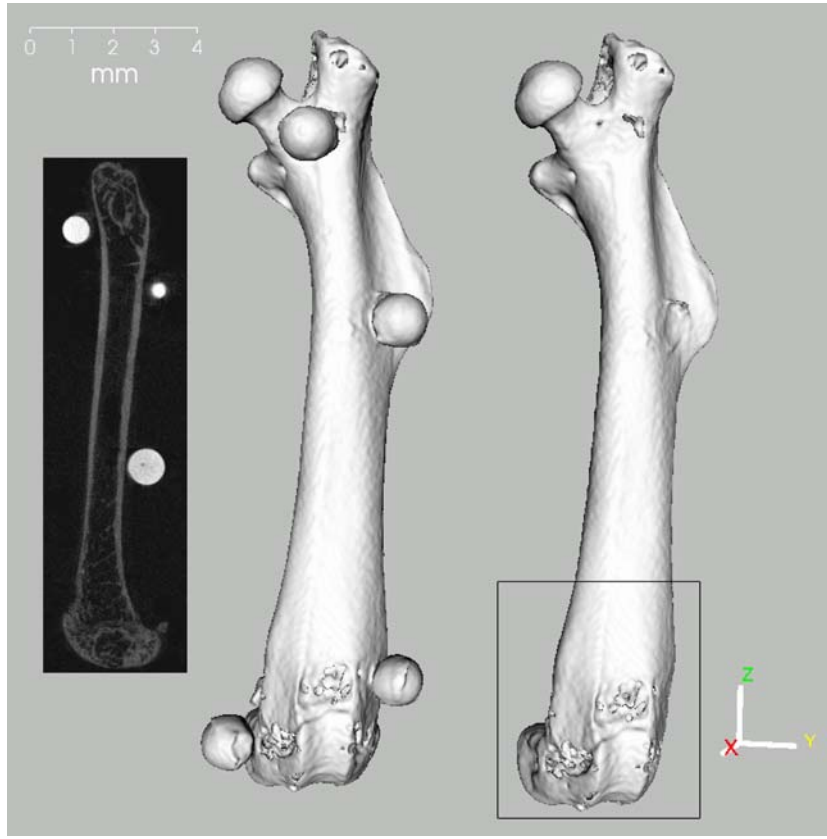


FIGURE 3. Spherical glass beads attached to the femur during micro-CT scanning (left 3D bone image) provided reference (fiducial) markers to evaluate the accuracy of the registration process. Prior to registration, the glass beads were removed from the images (right 3D bone image) so they would not influence the registration result. The distal region of the femur approximated by the box indicates the data used in the automated registration process. The inset (far left) illustrates the relative attenuation of the glass beads relative to the bone on a 2D slice.

Assessing Relative Accuracy

A standard method of assessing registration accuracy, named TRE, is based on a comparison of the resulting transform from registration, T_r , against a gold standard transform, T_{gs} , which was calculated over the position of all N voxels (excluding background), a_i , of the moving image,⁹

$$TRE^2 = \sum_i^N |T_{gs}(a_i) - T_r(a_i)|^2. \quad (4)$$

The gold-standard transform T_{gs} was determined for each image pair by finding the transformation of the centroids of corresponding fiducial marks (glass beads) using rigid body analysis methods.³⁴ Centroids were determined by averaging the spatial position of the surface nodes for each fiducial mark following isosurface extraction.

The TRE measure is limited by the accuracy of the reference gold-standard, therefore, the contribution of the errors in the gold-standard transformation was estimated. The root-mean-square (RMS) error of the

gold-standard transformation³⁴ provides a fiducial registration error (FRE) which can be used to estimate fiducial localization error (FLE)³⁵ by

$$FLE = \sqrt{\frac{N_f}{N_f - 2}} FRE, \quad (5)$$

where N_f is the number of fiducial points (i.e., six beads). The gold-standard errors were calculated for specific anatomic landmark locations, x , where $TRE(x)$ ⁹ is estimated by,

$$\langle TRE(x)^2 \rangle \cong \frac{\langle FLE^2 \rangle}{N} \left(1 + \frac{1}{3} \sum_{k=1}^3 \frac{d_k^2}{f_k^2} \right), \quad (6)$$

where for a given fiducial configuration with principal axis k , d_k is the distance of the target from the principal axis, and f_k is RMS distance of the fiducials from the principal axis. This calculation (Matlab, Mathworks, Natick, MA) provided TRE errors in the gold-standard transformation at specific anatomical locations.

Image Field Distortion

Image field distortion due to cone-beam tomographic acquisition in the micro-CT scanner can influence the gold-standard transformations, and have an important effect on registration accuracy. An estimation of the magnitude of image distortion was based on the relative positions of a constellation of eight fiducial markers embedded in polystyrene foam which was measured at five different Z-positions (i.e., perpendicular to source plane) in the micro-CT scanner field of view. The RMS error of the rigid body translation of the markers relative to a central Z-position³⁴ was the basis for the distortion estimate.

In vivo Image Registration

The image registration procedures were demonstrated on *in vivo* data, including one OVX and one sham-operated rat. These animals were part of a larger study used to understand the bone adaptation that occurs in an experimental model of osteoporosis.³ The 8 month old Wistar rats were *in vivo* micro-CT scanned (vivaCT 40, Scanco Medical, Bassersdorf, Switzerland) at 0, 4, 8 and 12 week post-OVX in the proximal tibia capturing 3.2 mm of the metaphyseal region (15 μ m nominal isotropic resolution). The proximal tibia was scanned instead of the distal femur (as per the *in vitro* data) because it is the most common region of analysis for *in vivo* micro-CT. The registration for each animal was performed between weeks 0–4, 4–8, and 8–12 resulting in transformations $[T_{0-4}]$, $[T_{4-8}]$, and $[T_{8-12}]$, respectively. Subsequently, all data was transformed into the coordinate system defined by week 0 (i.e., 12 week data was multiplied by $[T_{0-4}][T_{4-8}][T_{8-12}]$) so that local adaptations of the trabecular structure could be evaluated by superimposing the time series data. In addition to the entire proximal tibia data set, this procedure was applied to a central trabecular region ($1.95 \times 1.95 \times 1.50$ mm³). Datasets were typically 16×10^6 voxels for the full measured tibia, and 125×10^3 voxels for the central trabecular region (15 μ m voxel sizes for both cases). In contrast to the *in vitro* tests used for measuring registration performance, the registration of these *in vivo* data provide a qualitative assessment of rigid registration for bone that changes over time between subsequent measures.

RESULTS

On the distal femur of the *in vitro* data, although there were few readily identifiable and well-defined landmarks, three roughly picked landmarks per image were possible and sufficient such that all registrations converged. In the *in vivo* datasets where high period-

icity was encountered due to extracting a trabecular subvolume, the automated registration process was robust. Therefore, image resolution was normally reduced for landmark picking without jeopardizing the registration. Landmark picking was the only interactive component of the registration process.

The TRE measurements, representing registration accuracy relative to the gold standard, and computational efficiency were calculated by Eq. (4) for each registration similarity measure and each interpolator on the *in vitro* data (Fig. 4). The FRE for the six glass beads used for the gold-standard transformation was 7.0 μ m, and the corresponding FLE calculated by Eq. (5) was 8.6 μ m. For the three similarity measures tested, the registration accuracy for all tests was below 21.7 μ m (relative to a 32 μ m isotropic voxel size) and, although the mean differences between measures were not statistically significant, the mean squares measure trended toward the best result (9.4 μ m mean TRE). The CPU time per iteration for the three measures was 0.8s, 2.2s and 1.4s per iteration for the mutual information, normalized correlation and mean squares similarity measures, respectively. Of the three registration interpolators tested, the nearest neighbor result was significantly less accurate with a mean TRE of 19.7 μ m compared to the linear interpolator mean TRE of 10.6 μ m (*t*-test; $p < 0.04$). The TRE values for the linear and *b*-spline interpolators were not significantly different; however, the solution time for the *b*-spline interpolator was more than 10-fold that of the linear interpolator.

The convergence and solution time of the Mattes mutual information, normalized correlation or mean squares measures are presented in Fig. 5 for the linear interpolator with 300 and 100 iterations at downsizing factors 3.5 and 1.0, respectively. The computational time of the registration per iteration decreased as the downsizing factor was increased, where the mean time was 37.9s, 11.3s, 4.8s and 0.9s per iteration for downsizing factors of 1.0, 1.5, 2.0 and 3.5, respectively (standard deviations less than 0.2s/iteration). The spikes in the similarity measure values are artifacts due to the gradient descent optimizer jumping the minimum value, which is related to the dynamic increases or decreases in step size by factors 1.1 and 0.75, respectively. After a small number of iterations the step size is corrected, and the final registration transformation *T* was chosen based on the iteration with the smallest similarity measure value (not the final iteration which could correspond to a spike). Eliminating the spikes is possible by using a constant and conservatively small step size; however, this increases the required number of iterations to reach a final solution.

A representative registration pair (based on a median TRE result) was selected and the rate of TRE

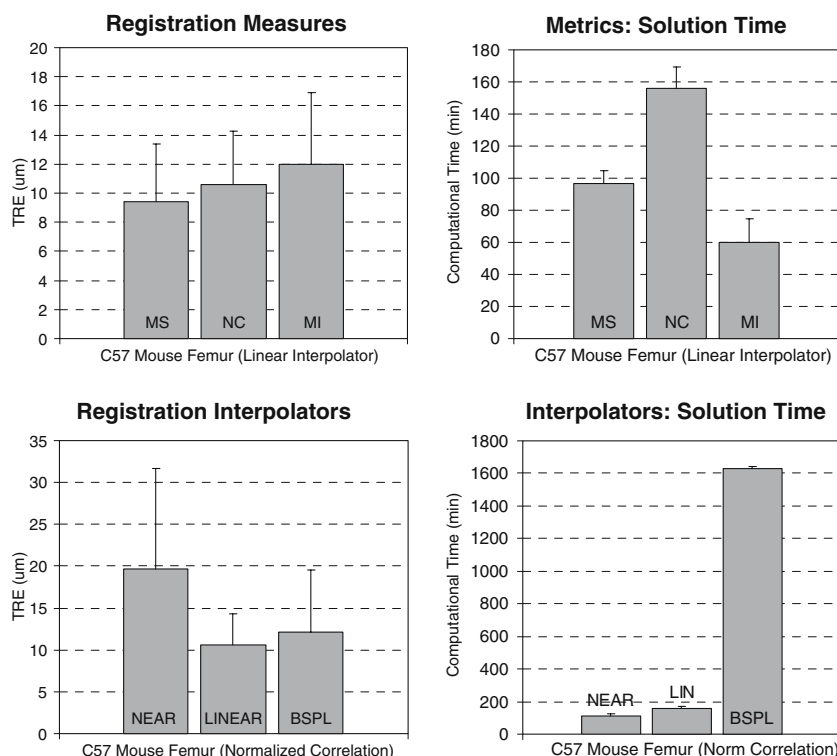


FIGURE 4. The target registration error (TRE) and solution time was calculated for three registration similarity measures (using a linear interpolator) and three interpolators (using a normalized correlation similarity measure) on the *in vitro* data. Similarity measures tested were mean squares intensity difference (MS), normalized correlation (NC) and Mattes mutual information (MI). Interpolators tested were a nearest neighbor (NEAR), linear (LINEAR) and *b*-spline (BSPL) interpolator. Bars represent averages and standard deviation error bars for each test ($N = 10$).

convergence was plotted by Eq. (4) as a function of (i) the registration similarity measure (keeping the downsizing factor constant at 1.0), and (ii) a function of different downsizing factors (Fig. 6; using the normalized correlation measure with a linear interpolator). The normalized correlation and mutual information measures had similar convergence patterns, but the mean squares measure was less stable (noting that the same initial approximate transformation was used for all three measures). The larger downsizing factor resulted in a faster rate of convergence.

Based on the TRE measurements from the mutual information measure combined with the linear interpolator (Fig. 4), the TRE was calculated explicitly at six arbitrary locations to illustrate local accuracy by Eq. (6), which was below $6 \mu\text{m}$ in each of the positions tested (Fig. 7).

Distortion of the 3D micro-CT image was measured at five locations within the cone beam reconstruction (Skyscan 1072) and an RMS value for each position was determined relative to when the glass-bead marker array was positioned in the central portion of the scanner field of view (Fig. 8). The RMS error increased proportionally as the glass-bead marker array was

positioned toward the top or bottom edges of the scanner field of view, and the maximum RMS error between the eight glass-beads was $15.1 \mu\text{m}$ indicating significant errors that could arise due to scanner distortion alone at the edges of the field of view.

The two *in vivo* micro-CT data sets consisting of four scans over a 12 week period were registered using a mutual information measure, linear interpolator, and a multi-resolution registration of 300 and 100 iterations at downsizing factors of 3.5 and 1.0, respectively. Subsequent to registration, representative slices from the OVX data set were extracted from the central trabecular region to illustrate the loss of trabecular bone over the 12 week period (Fig. 9). A series of sub-volumes ($50 \times 50 \times 50$ voxels) were extracted from the registered data sets for both the sham and OVX data and rendered. The sham data qualitatively demonstrates the ability of the 3D registration algorithm to extract the same region of trabecular bone from the four time series sets of data. The OVX visualizations exhibit the severe bone loss that occurs 12 week following the operation, and again qualitatively illustrates that consistent extraction of ROIs is possible using rigid registration even when significant changes to the local architecture occur.

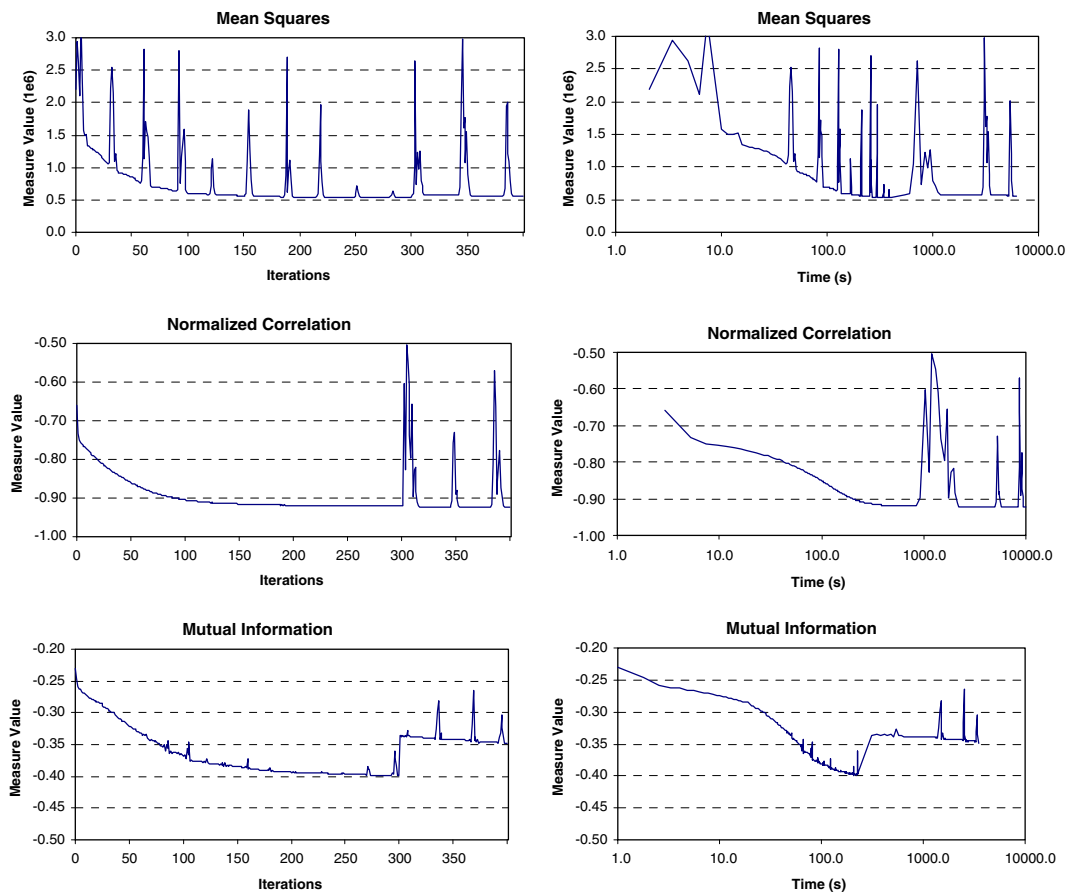


FIGURE 5. Convergence of the similarity measure value (mean squares, normalized correlation, or mutual information) as a function of the number of iterations (left column) and solution time (log 10 scale measured in seconds; right column) for each of the three registration similarity measures tested on the *in vitro* data. The first 300 iterations were performed with a scale factor of 3.5, and the final 100 iterations were performed at a scale factor of 1.0 (full resolution images). The discontinuity of the similarity measure value at 300 iterations is due to the change in scale factor.

DISCUSSION

All combinations of three registration similarity measures, including both intra- and inter-modal measures, with three interpolators were applied successfully to high-resolution *in vitro* micro-CT data, yet there were specific combinations that were more efficient and accurate based on the TRE gold-standard. Subsequently, registration of *in vivo* micro-CT data was performed to demonstrate its applicability for a rat model of experimental osteoporosis and for application to temporal data where significant bone change occurs.

The micro-CT image registration approach used in this study was semi-automated, requiring two distinct steps: (i) interactive selection of corresponding landmarks to provide an initial approximate transformation, and (ii) automated registration. The user interaction component of selecting landmarks was based on isosurface renderings²³ of the micro-CT data

and was a simple and robust process. A knowledge of the anatomy of the specimen was not necessary, only the ability to identify common landmarks between image pairs. An increase in efficiency was achieved by rendering reduced resolution images prior to isosurfacing. The choice of isosurface value did not impact the accuracy of the landmark selection process. It should be noted that the subsequent registration procedure was applied to raw micro-CT data and was independent of the image processing applied for the visualization techniques to select landmarks. All of the initial approximate transformations determined for the *in vitro* and *in vivo* data led to convergence of the similarity measures when using the multi-resolution registration approach.

The selection of the best combination of similarity measure, interpolator and multi-resolution scheme is complex, and the TRE gold-standard measure derived from the *in vitro* data provides important insight (Fig. 6). Clearly, the accuracy would not be as good

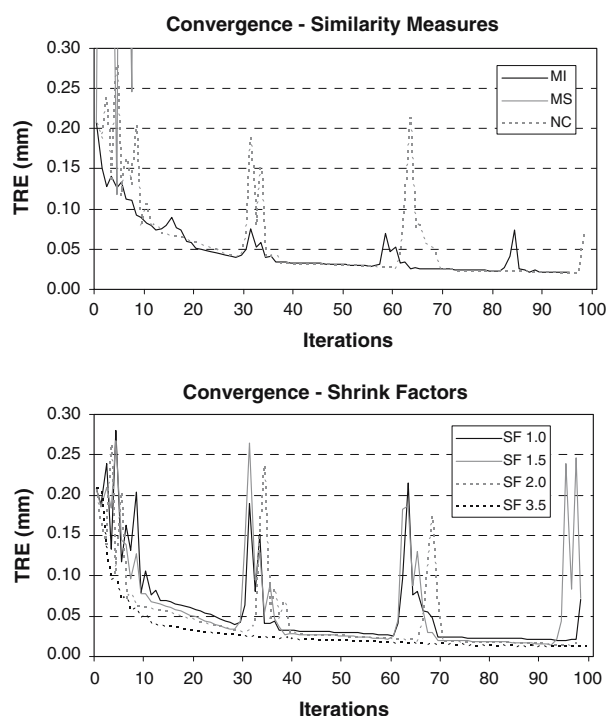


FIGURE 6. Convergence of the TRE value for a specific registration pair. For all tests the same initial guess and a linear interpolator was applied. The relative convergence of the three similarity measures (top) using a downsizing factor of 1.0 shows convergence of the normalized correlation and mutual information measures, but divergence of the mean square measure. The convergence of TRE values as a function of shrink factor (bottom) using the normalized correlation measure demonstrates a faster rate of conversion as the downsizing factor is increased to 3.5.

for *in vivo* registration where growth and bone adaptation can be expected; however, the TRE measure provides a lower bound estimate of accuracy. Using the TRE, selection of the best interpolator was relatively straightforward as the linear and *b*-spline interpolators demonstrated improved accuracy over the nearest neighbor interpolator. However, based on efficiency, the linear interpolator was better suited than the cubic *b*-spline interpolator for high-throughput registration because of the 10-fold increase in computational time.

The selection of an optimal registration similarity measure based solely on TRE accuracy is not appropriate because all three measures performed well on the *in vitro* data (Fig. 4). In terms of solution efficiency, mutual information provided the fastest results with respect to CPU per iteration and solution convergence (Fig. 5) despite the trend towards least accurate TRE measurements (Fig. 4). However, caution must be exercised when comparing efficiency of similarity measures because there are many possible parameter settings that can be adjusted to ‘tune’ the registration. For example, the performance of the mutual infor-

mation metric is sensitive to the number of spatial samples used to calculate the similarity measure, and although use of 20% of the total voxels here produced good results, it is probable that other data with increased noise would require more samples (i.e., >20%) resulting in significantly increased solution time (but ensuring a smooth optimization process). To illustrate this point, a short test was performed where we increased the number of spatial samples to 100%, and this resulted in a 5-fold increase in CPU time per iteration, but with fewer optimizer spikes. Another consideration affecting total solution time is the optimizer stopping condition. Although a fixed stopping condition was used for each similarity measure for the purpose of straightforward comparisons in this study, it is recommended that a convergence criterion be used in practice. The selection of an appropriate convergence criterion will depend on the type of similarity measure and desired registration accuracy, and will directly impact total registration solution time. So, selection of the best similarity measure purely based on solution speed is difficult, particularly since normalized correlation also provided reasonable results. It would seem that mean squares is the least favorable method as it was not well behaved (i.e., solution did not converge) for registration of the full resolution images (Fig. 6), although application of an initial downsizing factor of 3.5 applied in multi-resolution registration mode ensured a well behaved optimization for all the measures tested. Alternatively, another factor that distinguishes mutual information and normalized correlation measures is that the former is intended for inter-modal registration and the latter for intra-modal registration. On the *in vitro* data, either method performs well; however, for *in vivo* data the inter-modal method (mutual information) may be more appropriate as it is less sensitive to changes in image intensities that will occur due to bone adaptation.

For a given selected measure and interpolator, the most dramatic impact on registration efficiency was the application of a multi-resolution approach, where the time per iteration improved from 37.9s/iteration at a downsizing factor of 1.0 compared to 0.9s/iteration at a downsizing factor of 3.5. Not only was the convergence of the measure faster using a large downsizing factor (Fig. 5), but the accuracy of the registration using the higher downsizing factor was surprisingly reasonable in the first 100 iterations tested (Fig. 6). Based on the TRE results, it appeared that a multi-resolution approach using a linear interpolator and Mattes mutual information measure can provide sub-voxel accurate registration results. The absolute registration errors at the local error level based on TRE measures at the distal end of the femur (Fig. 7), a particular area of interest for biomedical research, was

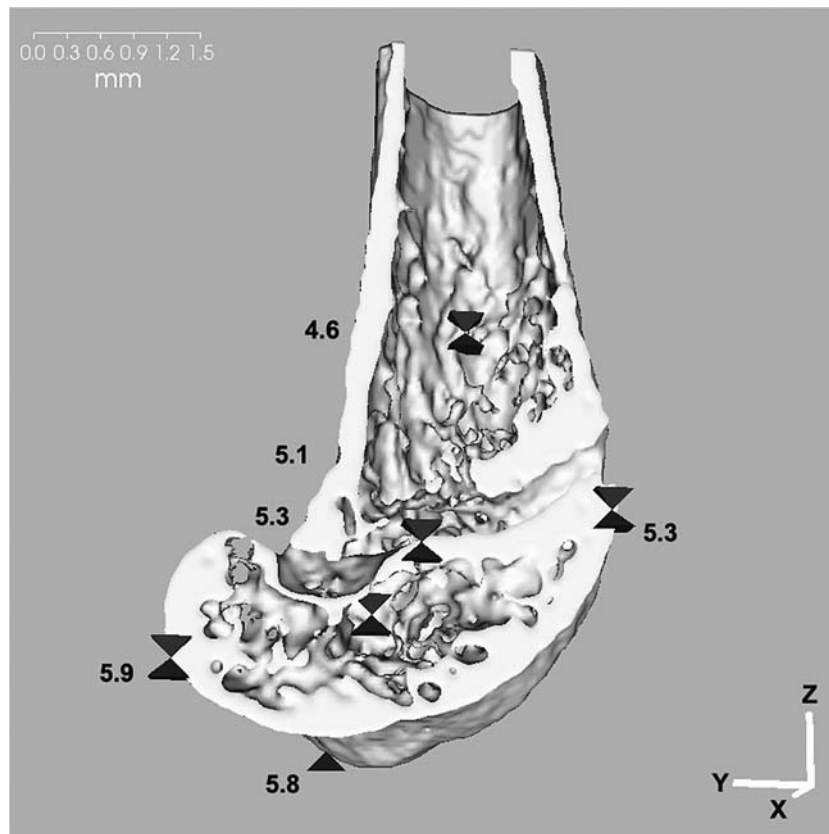


FIGURE 7. The target registration error (TRE) measured in micrometers relative to the gold standard transformation based on fiducial points (glass beads) was calculated using Eq. (6) at six arbitrarily chosen anatomical landmarks to provide an estimate of local registration error.

between 4–6 μm compared to an image resolution of 32 μm .

The registration of the *in vivo* data demonstrated that the rigid registration methods could qualitatively identify consistent ROI in temporal data series despite large changes in the internal micro-architecture (approximately 72% decrease in bone volume ratio) as illustrated in the sub-volumes extracted (Fig. 9). The 2D slices from the temporal series of data demonstrated the severe loss of trabecular structure that occurred within 12 week of OVX that is consistent with other OVX studies,^{20,21,41} and the 3D rendered data provided an excellent qualitative assessment of the ability to select consistent ROIs. As expected, little structural changes occurred in the sham animal whereas severe micro-architectural changes appear in the OVX series. The degree of bone loss that occurred in the OVX rat is larger than would be expected in clinical applications of micro-CT,^{2,18} nevertheless identification of consistent ROI was possible even in this difficult dataset. The impact of registration for identifying consistent ROIs and hence improved precision of morphometric parameters for *in vivo* applications in both animal and clinical studies may be

significant, particularly when small temporal changes occur.

There are several aspects of micro-CT registration that should be considered to optimize performance and accuracy. First, if possible, registration of image pairs should be performed on the gray-scale data rather than segmented data due to the possible loss of image information. However, the mutual information measure is capable of registering segmented images, for example, when compiling anatomical atlas maps.⁶ Second, for a given image pair, the selection of the fixed and moving images is not arbitrary and the decision may impact the efficiency of the registration process if volume clipping is not employed.³² Without volume clipping, it is recommended that the fixed image be defined as the image with the least number of voxels because fewer computations per iteration are required to interpolate into the moving image coordinate system for calculation of the registration measure. In *in vivo* experimental protocols, it may be desirable to perform a high resolution scan at the termination of the experiment when possible (compared to earlier *in vivo* scans in the protocol where radiation exposure may have been a concern^{1,10,31}), thus the final high

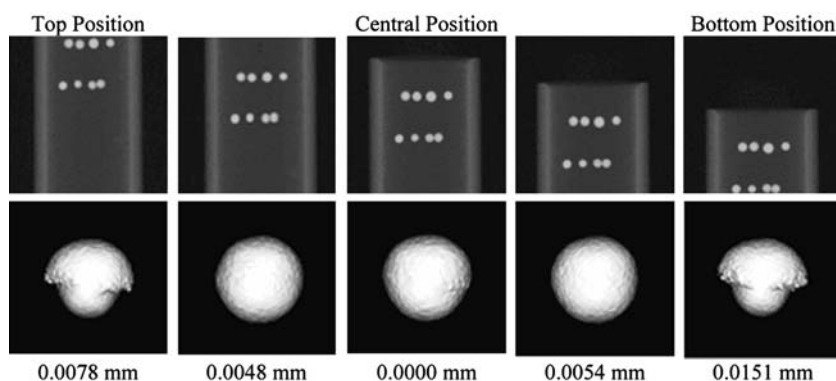


FIGURE 8. Distortions of the image occurs depending on the position of the specimen (8 glass-bead marker array in this case) relative to the micro-CT scanner field of view. The 2D projection images, a 3D reconstruction of one representative bead, and the root-mean-square (RMS) based on the rigid body transformation error calculated with respect to the central position in the micro-CT scanner are presented for five positions. The distortion increases as the glass-bead marker array are positioned further from the central micro-CT field of view as illustrated qualitatively by the 3D reconstructions and quantitatively by the RMS fit error. Minimizing micro-CT distortion errors in follow-up measurements can be achieved by ensuring consistent specimen positioning within the field of view.

resolution scan should be defined as the moving image to which all of the previously acquired smaller *in vivo* measurements are registered. The registration of mismatched image sizes is possible because there is no requirement for perfect correspondence of ROI between image pairs, only that there is some degree of overlap between the images. Third, distortions in the micro-CT images may occur at the margins of the scanner field of view for cone-beam reconstructions as was demonstrated (Fig. 8). Minimizing the number of

slices per reconstruction (i.e., moving closer to a fan beam reconstruction) would decrease the non-linear distortions; however, the reconstruction approach is intrinsically designed into each scanner and typically cannot be controlled by the user. Therefore, it is recommended that for *in vivo* measurements the specimen be placed as consistently as possible in the same position within the scanner to reduce non-linear image distortions, which could be incorrectly interpreted as bone adaptations over time. Lastly, visualizing the

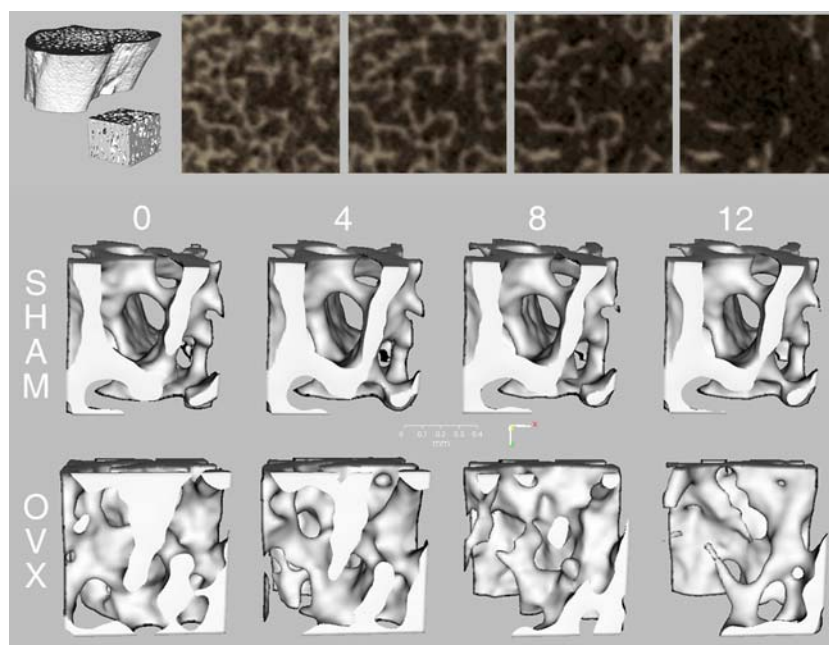


FIGURE 9. Image registration of *in vivo* micro-CT data from measurements at 0, 4, 8 and 12 weeks post-ovariectomy (OVX) (top). The four 2D slices shown were extracted from the 3D registered image data (mutual information, linear interpolator), and indicate good correspondence between images. All registered data sets were transformed into the same coordinate system defined by the measurement at week 0. Visualizations of a sub volume of *in vivo* micro-CT data at weeks 0, 4, 8 and 12 for a sham and an ovariectomy (OVX) operated rat are shown. Consistent regions of interest from the central portion of the metaphyseal bone in the proximal tibia were extracted.

results of registration methods is recommended whenever possible as use of automated methodologies may lead to unexpected errors.

The intrinsic limitation of rigid registration is that the six degrees of freedom transformation cannot account for spatial non-linear changes in the micro-architectural structure (either due to modeling, remodeling, growth or micro-CT image distortion). This limitation may be mitigated by using smaller ROIs where adaptations are more closely approximated as linear. One possible strategy to account for non-linear bone changes (growth or resorption) in longitudinal measurements is to mask regions of significant skeletal adaptation prior to registration;³⁹ however, this approach may introduce a bias due to user judgment. Ideally, longitudinal measurements should assess a large ROI to provide a better overview of the adaptive process. A possible approach is to use non-linear registration methodologies (i.e., deformable registration), and these methods are currently being investigated,⁵ however, they are significantly more complex and require more computational power. Rigid registration is an efficient approach for high-throughput research and it is expected to play an important role in research using *in vivo* micro-CT technology.

In conclusion, using Mattes mutual information with a linear interpolator and a multi-resolution scheme provided efficient and accurate registration of micro-CT data. The availability of registration for *in vivo* micro-CT experiments will extend analysis of temporal data by providing important additional information for understanding the micro-structural adaptations that occur in bone. Image registration will be a fundamental technique for *in vivo* micro-CT either in experimental or clinical studies for diagnosing bone diseases and monitoring the efficacy of interventions.

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