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A non-rigid registration method for serial lower extremity hybrid SPECT/CT imaging

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Abstract

Small animal X-ray computed tomographic (microCT) imaging of the lower extremities permits evaluation of arterial growth in models of hindlimb ischemia, and when applied serially can provide quantitative information about disease progression and aid in the evaluation of therapeutic interventions. The quantification of changes in tissue perfusion and concentration of molecular markers concurrently obtained using nuclear imaging requires the ability to non-rigidly register the microCT images over time, a task made more challenging by the potentially large changes in the positions of the legs due to articulation. While non-rigid registration methods have been extensively used in the evaluation of individual organs, application in whole body imaging has been limited, primarily because the scale of possible displacements and deformations is large resulting in poor convergence of most methods. In this paper we present a new method based on the extended demons algorithm that uses a level-set representation of the body contour and skeletal structure as an input. The proposed serial registration method reflects the natural physical moving combination of mouse anatomy in which the movement of bones is the framework for body movements, and the movement of skin constrains the detailed movements of the specific segmented body regions. We applied our method to both the registration of serial microCT mouse images and the quantification of microSPECT component of the serially hybrid microCT-SPECT images demonstrating improved performance as compared to existing registration techniques.

Keywords

Non-rigid registration; serial mouse registration; demons algorithm; large displacement

1 Introduction

Peripheral arterial disease (PAD) is a major clinical problem afflicting 8–12 million Americans that causes significant morbidity and mortality. The recent introduction of gene therapies and drug delivery systems for treatment of various diseases has caused a remarkable need for non-

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invasive imaging approaches to evaluate and track the progress of these therapies. The pathophysiology of PAD and developing therapeutic interventions can be evaluated by hybrid single photon emission computed tomographic (SPECT) and X-ray computer tomography (CT) [1,2,3,4].

The fusion strategy allows for the high resolution anatomical vascular imaging with CT in combination with functional indices from SPECT or alternatively positron emission tomographic (PET) imaging system. MicroCT, when used in combination with radiotracer based imaging, is expected to play an important role in understanding the origins and the progress of diseases, as well as in evaluating the efficacy of therapeutic interventions. Dedicated hybrid microSPECT/CT and microPET/CT imaging systems have been developed to image small rodents, and can be used to define the pathophysiology of PAD. The non-rigid registration of microCT with microSPECT or microPET image sets allows for accurate registration and quantification of serial images.

Our proposed analysis method in this paper deals with the non-rigid registration of the microCT images from hybrid microSPECT/CT image sets with the long-term goal of developing image methodology for the *in vivo* evaluation of the functional/metabolic changes derived from microSPECT with structural changes from microCT in models of hindlimb ischemia. These approaches also have implications for evaluation of PAD in patients with similar hybrid imaging systems.

Image registration is the process of geometrically aligning two or more images. Typically, the new altered image is aligned to the reference image by a spatial transformation. Image registration is often used as a preliminary step before other image processing applications. Non-rigid registration methods allow for the mapping of images containing large deformations (e.g. inter-subject brain registration). Most work in this area has focused on brain registration, where there are a large number of publications [20,21,22,27]. In addition, these types of methods have been applied to other single “organ” cases such as the heart and the prostate [23,24,29,30]. Our target application for image registration is that of the whole body, or more accurately in this case lower extremities of rodents. However, a solution to this problem has many potential applications, including the translation of this approach to peripheral CT angiograms in patients. A similar application relates to the quantification of functional/metabolic changes over time assessed with several hybrid imaging modalities such as PET/CT and SPECT/CT. Another emerging application is the use of image registration as a way to determine the phenotype of genetically altered mice by comparing the anatomy of transgenic mice to a matched group of wildtype homologues [25].

In many cases, longitudinal comparisons between each subject’s baseline image and that subject’s follow-up images provide useful information on disease progression. Serial registrations have been used to identify anatomical differences over time in rheumatoid arthritis study [37] and the cross-sectional changes of the brain in different group of mice [38].

A challenge in these broader applications is the presence of potentially large displacements located close to joints in the body which create convergence problems for many standard methods (as illustrated in our results section). The regions close to limb joints contain many vascular structures whose relative positions vary substantially from subject to subject even when extra care is taken to implement standard positioning and a standardized imaging protocol. This is particularly true in our application of serial 3-dimensional microSPECT/CT imaging for the purpose of evaluating arteriogenesis/angiogenesis associated with peripheral arterial disease of the lower extremities. In this application, microCT angiography is used to evaluate lower extremity arteriogenesis, while registered microSPECT images provide a

quantitative index of changes in either tissue perfusion or critical radio-labeled molecular markers of the underlying biological process [36].

Our previous work [16] and Li *et al.* [17,18] used a robust point-based method (RPM) with inputs of two cloud sets of points from bone and body surface contours to provide a good initial condition for an intensity-based registration as a next step after the RPM. Little *et al.* [19] proposed a method that allows the rigid deformation of identified objects, while the remainder deforms non-rigidly. They registered vertebrae using rigid transformation, and they registered a neck using smoothing deformations based on the rigid transformation. One of the limitations of this method is that all objects for the rigid transformation should be identified. Prior knowledge about bone correspondence between the reference and new images are indispensable for this method. Explicit articulated models [12,28] have also been used for the serial registration of lower-limb mouse images and human hand images.

Recently, some methods for registration of whole-body small animal image have appeared in the literature. Kovacevic *et al.* [39] proposed a hierarchical anatomy tree of a mouse and its hierarchical piece-wise affine registration, in which a connected part of skeletal structures in a lower level is initialized by the registration result of another skeletal structures in a higher level. In order to set up the hierarchical tree of skeletal structures, manual or semi-automatic labeling of the images was required using prior anatomical knowledge. Based on the hierarchical registration, Baiker *et al.* [40] adopted an iterative closest point (ICP) rigid registration for mice skeletons in each anatomical layer of CT images. They extended this method to approximating organs around the skeletons using a local shape context (the positional relationship of neighboring points) and a thin-plate-spline (TPS) [42]. Chaudhari *et al.* [41] and Joshi *et al.* [44] also tried to estimate the internal organs of a given mouse surface image from a 3D mouse atlas based on the manually identified landmarks using a 3D harmonic volume mapping [41] or an elastic volume warping from the landmarks [44]. Xiao *et al.* [46] proposed a modified three-dimensional shape context matching method for aligning rat skeletons from CT image. They combined the positional relationship of neighboring points with curvature information for correspondences of points to avoid any joint definition on the skeleton. In related work, Martín-Fernández *et al.* [43] proposed an articulated registration method for human hands in conventional 2D X-ray images. They combined a rigid transform for bones with a landmark-based elastic registration for soft tissues around bones. The extent of elasticity of the soft tissues was decided on the distance from the bones using a distance transform from the centerlines of bones.

In this work, we present a method based on a combined surface registration using both bone and body surfaces. The alignment of the bone surfaces is a natural first step as they “carry” the rest of the soft tissues along. The soft tissue surface registration enables the use of additional constraints to bound the registration. The combination of these different surfaces into a single method is a key challenge. From Figure 1, one can easily see that the motions generated by the movement of bones are different from the motions by the movement of soft tissues despite their proximity. In this paper, a three-step serial registration method is presented. The first step is a bone surface registration, and the second step is a body surface registration based on the result of the first step. The third step is an intensity based registration to refine the results from the previous steps. We evaluate our method both in terms of registration accuracy and in terms of accuracy of quantification of microSPECT results. In both cases our method demonstrates improved performance over alternative registration techniques.

2 Methods

Figure 2 shows the brief block diagram of the proposed method. This method is a three-step serial registration composed of a bone surface registration, a skin surface registration and an

intensity registration. After initially executing the bone surface registration (f_1), we execute the skin surface registration (f_2) based on the initial bone surface registration result. Then, we refine the image intensities for the soft tissue between the bone and skin by the final intensity registration (f_3). We combine these three registrations as one registration using concatenation ($f_{total} = f_3 \circ f_2 \circ f_1$). The proposed serial registration method reflects the natural physical moving combination of mouse anatomy in which the movement of bones is the framework for body movements, and the movement of skin constrains the detailed movements of the specific segmented body regions. We first review extended demons method for single surface mapping such as a bone surface. Then we describe each step in the proposed method.

2.1 Extended Demons Registration

Using the demons concept from Maxwell's demons algorithm in thermodynamics, Thirion [6] considered the non-rigid image matching process as a diffusion process. The theory behind this algorithm is that the object boundary can change its shape depending on the position of the demons within the image domain. The demons' forces, which deform the object shape, are generated to reduce the disparity between the source and the target images. Equation (1) shows Thirion's demons algorithm based on the optical flow equation. Here, $\mathbf{r}(\mathbf{X})$ is the reference image, $\mathbf{m}(\mathbf{X})$ is the moving image and $\mathbf{v}(\mathbf{X})$ is the displacement.

$$\mathbf{v}(\mathbf{X}) = - \frac{(\mathbf{m}(\mathbf{X}) - \mathbf{r}(\mathbf{X})) \nabla \mathbf{r}(\mathbf{X})}{\|\nabla \mathbf{r}(\mathbf{X})\|^2 + (\mathbf{m}(\mathbf{X}) - \mathbf{r}(\mathbf{X}))^2} \quad (1)$$

The local displacement field as a transformation model $\mathbf{T}$ is updated at each voxel $\mathbf{X}$ by $\mathbf{T}(\mathbf{X}) = \mathbf{X} + \mathbf{v}(\mathbf{X})$ iteratively.

Many studies improved the original demons algorithm by increasing the speed of convergence [8,13,14], and by enhancing versatility and accuracy [8,10,9,15]. Recent diffeomorphic demons [33] requires more rigorous regularization for both a differentiable mapping and a differentiable inverse mapping in diffeomorphism. For stronger convergence performance, the extended demons registration method [8] takes advantage of the level-set representation of the object boundary using a distance map based on the original demons algorithm (Figure 3 (a)). In the level-set representation of the shape, distances inside the object boundary are denoted by negative values, and distances outside the boundary are denoted by positive values. The employment of the distance map in a registration process was previously presented by Wildeman *et al.* [45] in their non-deformable registration work between 2D photograph image and 3D CT image.

The extended demons algorithm utilizes the distance map around the shape border instead of intensity and results in improved convergence even though two objects are not overlapped sufficiently. Besides, in the case of a segmented binary three dimensional shape without intensity, the distance map from the binary shape plays a role similar to that of consistent intensities, and then demons forces occur in all voxels in addition to the object boundary (in case of the original demons registration between binary objects, the intensity difference between the two objects does not produce any flow). Thus, the extended demons registration algorithm can achieve much higher convergence speed with better confidence in binary volume registration. However, variations of shape to tangential direction cannot be well described by extended demons registration algorithm since level-set representation of shape by a distance map captures only the normal direction to the shape border. The extended demons registration method also has a limitation in that it does not take advantage of any feature information based on the similarity between the two objects. Thus, the registration results may not be satisfactory when one or both of the objects have complicated, twisted regions like Figure 3 (b) and (c). In order to overcome this limitation, Suh and Wyatt [10] added a feature matching algorithm using

centerline analysis to the extended demons algorithm, and Lüthi *et al.* [9] included a curvature term to guide the registration process for corresponding similar curved regions. In this paper, we used a symmetric version [7] of the extended demons method to reduce local minima as below.

$$\mathbf{v}(\mathbf{X}) = - \frac{2(\mathbf{m}(\mathbf{X}) - \mathbf{r}(\mathbf{X}))(\nabla \mathbf{r}(\mathbf{X}) + \nabla \mathbf{m}(\mathbf{X}))}{\|\nabla \mathbf{r}(\mathbf{X}) + \nabla \mathbf{m}(\mathbf{X})\|^2 + (\mathbf{m}(\mathbf{X}) - \mathbf{r}(\mathbf{X}))^2} \quad (2)$$

2.2 First Step: Registration of the Bone Surface

Although bones are rigid objects, rigid registration methods for bone surfaces are obviously inappropriate because of the articulated joints such as the hip, the knee, and the ankle joints in mice. For example, the relative orientation of the proximal and the distal leg is highly dependent on the state of the knee joint and can vary substantially between subjects even when extra care is taken to implement a standardized imaging protocol. While a simple piecewise rigid model can be applied, such models suffer from discontinuities at the motion boundary leading to both folding and stretching [12]. As articulated structures, bones are completely enclosed in the skin. Since this anatomical constraint will be included in the calculation of displacements in the next step, the bone registration is freely performed through an implicit surface registration method as follows. Figure 4 shows the block diagram of the serial registration of bone surface, skin surface and image intensity registrations. From each reference and moving CT image of a given mouse, binary bone images and binary skin images are segmented through intensity differences by thresholding and clustering (K-means clustering with 3 classes). The bottom images in Figure 5 (a) and (b) shows examples of the bone and skin surfaces segmented from their graylevel images (the top images in Figure 5 (a) and (b)). The level-set represented bone images are generated by a distance map from the binary reference bone image and the binary moving bone image respectively (Figure 6). Using equation (3) of the extended demons algorithm, the deformation between the two level-set represented bone images is calculated.

$$\mathbf{v}_{1B}(\mathbf{X}) = - \frac{2(\mathbf{m}_B(\mathbf{X}) - \mathbf{r}_B(\mathbf{X}))(\nabla \mathbf{r}_B(\mathbf{X}) + \nabla \mathbf{m}_B(\mathbf{X}))}{\|\nabla \mathbf{r}_B(\mathbf{X}) + \nabla \mathbf{m}_B(\mathbf{X})\|^2 + (\mathbf{m}_B(\mathbf{X}) - \mathbf{r}_B(\mathbf{X}))^2} \quad (3)$$

Here, $\mathbf{r}_B(\mathbf{X})$ is the reference image represented by level-set from the bone boundary, $\mathbf{m}_B(\mathbf{X})$ is the moving image represented by level-set from the bone boundary, and $\mathbf{v}_{1B}(\mathbf{X})$ is the displacement between $\mathbf{r}_B(\mathbf{X})$ and $\mathbf{m}_B(\mathbf{X})$.

2.3 Second Step: Registration of the Skin Surface

After the registration of bone surface in the first step, the moving skin image is deformed with the displacement of the bone surface registration result (the right image of Figure 5 (d)). Figure 5 (d) displays the deformation results of both bone and skin surfaces by the only bone surface registration in the previous step (block “B” in Figure 4). The skin surface deformed by the bone registration result is inaccurate. This is because the bone’s motion is different from the skin’s motion even though they are close to each other. In order to correct the inconformity of the skin motion, we execute the extended demons algorithm again, this time using the skin surfaces between the reference skin image and the moving skin image (which is deformed by the previous bone surface registration result). In the same way as the bone registration, the level-set represented skin images are generated by distance maps from the binary reference skin image and the binary moving skin image respectively (Figure 7). Using equation (4), we calculate the displacement between the two level-set represented skin images.

$$\mathbf{v}_{2S}(\mathbf{X}) = - \frac{2(\mathbf{m}_{1S}(\mathbf{X}) - \mathbf{r}_S(\mathbf{X}))(\nabla \mathbf{r}_S(\mathbf{X}) + \nabla \mathbf{m}_{1S}(\mathbf{X}))}{\|\nabla \mathbf{r}_S(\mathbf{X}) + (\nabla \mathbf{m}_{1S}(\mathbf{X}))\|^2 + (\mathbf{m}_{1S}(\mathbf{X}) - \mathbf{r}_S(\mathbf{X}))^2} \quad (4)$$

Here, $\mathbf{r}_S(\mathbf{X})$ is the reference image represented by level-set from the skin boundary, $\mathbf{m}_{1S}(\mathbf{X})$ is the moving image represented by level-set from the skin boundary (deformed by the result ($\mathbf{v}_{1B}(\mathbf{X})$) of the first step), and $\mathbf{v}_{2S}(\mathbf{X})$ is the displacement between $\mathbf{r}_S(\mathbf{X})$ and $\mathbf{m}_{1S}(\mathbf{X})$. In this second step, bone surfaces are registered again simultaneously (during the same iteration) with the skin surface registration using equation (5).

$$\mathbf{v}_{2B}(\mathbf{X}) = - \frac{2(\mathbf{m}_{1B}(\mathbf{X}) - \mathbf{r}_B(\mathbf{X}))(\nabla \mathbf{r}_B(\mathbf{X}) + \nabla \mathbf{m}_{1B}(\mathbf{X}))}{\|\nabla \mathbf{r}_B(\mathbf{X}) + (\nabla \mathbf{m}_{1B}(\mathbf{X}))\|^2 + (\mathbf{m}_{1B}(\mathbf{X}) - \mathbf{r}_B(\mathbf{X}))^2} \quad (5)$$

Here, $\mathbf{m}_{1B}(\mathbf{X})$ is the moving image represented by level-set from the bone boundary (deformed by the result ($\mathbf{v}_{1B}(\mathbf{X})$) of the first step), and $\mathbf{v}_{2B}(\mathbf{X})$ is the displacement between $\mathbf{r}_B(\mathbf{X})$ and $\mathbf{m}_{1B}(\mathbf{X})$.

During each iteration of the extended demons algorithm the two displacement fields ($\mathbf{v}_{2B}(\mathbf{X})$ and $\mathbf{v}_{2S}(\mathbf{X})$) are spatially combined in every iteration by equation (6) within the block “A” in Figure 4.

$$\mathbf{v}(\mathbf{X}) = \mathbf{w}_B(\mathbf{X})\mathbf{v}_{2B}(\mathbf{X}) + \mathbf{w}_S(\mathbf{X})\mathbf{v}_{2S}(\mathbf{X}) \quad (6)$$

Where $\mathbf{w}_B(\mathbf{X})$ and $\mathbf{w}_S(\mathbf{X})$ are weight factors defined as:

$$w_B(x_k) = \begin{cases} 1 & \text{if } d_B(x_k) \leq 0 \text{ and } d_S(x_k) < 0 \\ 0 & \text{if } d_B(x_k) > 0 \text{ and } d_S(x_k) \geq 0 \\ \frac{|d_S(x_k)|}{|d_B(x_k)| + |d_S(x_k)|} & \text{if } d_B(x_k) > 0 \text{ and } d_S(x_k) < 0 \end{cases} \quad (7)$$

$$w_S(x_k) = \begin{cases} 0 & \text{if } d_B(x_k) \leq 0 \text{ and } d_S(x_k) < 0 \\ 1 & \text{if } d_B(x_k) > 0 \text{ and } d_S(x_k) \geq 0 \\ \frac{|d_B(x_k)|}{|d_B(x_k)| + |d_S(x_k)|} & \text{if } d_B(x_k) > 0 \text{ and } d_S(x_k) < 0 \end{cases} \quad (8)$$

Here $|d_B(x_k)|$ refers the absolute value of the signed distance value and k is the voxel index. As shown in Figure 8, the displacement of the bone registration $\mathbf{v}_{2B}(\mathbf{X})$ is exclusively used within the bone region, and the displacement of the skin registration $\mathbf{v}_{2S}(\mathbf{X})$ is exclusively used outside the skin region. The displacement of the soft tissue between bone and skin is calculated by interpolation depending on the distance from both the bone and skin. In order to reduce the abrupt change of displacements, the standard deviation of the Gaussian smoothing filter within the extended demons algorithm is set to a large value ($\sigma = 2$ voxels). Figure 5 (e) shows a wireframe rendering example of this second step's registration result. The green bone and skin surfaces are the warped (post-registration) versions of the yellow bone and skin surfaces in Figure 5 (d). As one can see in Figure 5 (e), the bone and skin surfaces are well registered respectively through the first and second steps even though the motion changes by the bone and skin are different from each other (as indicated in Figure 1).

2.4 Third Step: Registration of Graylevel Intensity Image

After finishing the registration of bone and skin surfaces, soft tissues between the bone and skin are registered through this third step. In the third step, the symmetric version of the original demons algorithm [6] with histogram matching is used for the image intensity registration instead of the extended demons algorithm. The moving graylevel intensity image is deformed with both the displacement (f_1) of the bone surface registration result and the displacement (f_2) of the skin surface registration result as shown in Figure 4. The deformed moving graylevel intensity image and the reference graylevel intensity image are used as inputs of this third step of registration. Figure 5 (f) shows the resulting graylevel intensity image of the third step, which is deformed through the first (f_1), second (f_2) and third (f_3) displacements.

For registering the articulated hindlimbs of mice, we perform the surface registration following the natural “anatomical” constraints of first registering the bone surfaces – which literally anchor the rest of the body and then refining this by introducing skin surface information. The result of the surface-based registration is then augmented by an intensity based non-rigid registration as before. In each bone and skin surface registration, we adopt an implicit surface registration method which relies on a distance-map parameterization of the bone and skin surfaces.

2.4.1 Implementation Details—Three resolution layers were utilized in each step from the coarsest to the finest layer to search the displacement in the 3D data set. Iteration numbers for each layer were set to 200, 200 and 30 from the coarsest to the finest layer, respectively. The computational time for the whole three-step registration process was around 38 minutes per each study using Intel core Quad 2.5GHz processor with 8GB memory.

3 Results

We present here experimental results demonstrating the performance of our proposed algorithm. We first describe our general experimental procedures (Section 3.1) This is followed by a description of our accuracy validation experiments (Section 3.2), where we quantify raw registration accuracy and an evaluation of the ability of our algorithm to quantify serial SPECT images (Section 3.3); this last step being the ultimate goal of this work.

3.1 Experimental Procedures

3.1.1 Image Acquisition—The data used for this evaluation were derived from a serial microSPECT/CT imaging study of peripheral angiogenesis and arteriogenesis in response to ischemic injury. All animal experiments were performed according to the guiding principles of the American Physiologic Society and approved by the Institutional Animal Care and Use Committee. The imaging protocol consisted of first baseline imaging. Next, a ligation of the left femoral artery was performed to induce ischemic injury in the left hindlimb, with the right hindlimb functioning as a paired control. Following this procedure, the mice were then imaged at 3, 7 and 14 days to evaluate the effects of the induced ischemia.

When the mouse was positioned in the scanner, a technologist attempted to position the mouse approximately in the same posture as in the first acquisition. Despite this, the relative posture of the mouse is still different from the first acquisition, and the consequent arrangement of bones is altered from the arrangement at baseline. We also note that the experiments took place over a period of two weeks so the reproducibility of the mouse placement could only be approximate at best. (See Figure 9).

Imaging was performed using a hybrid micro-SPECT/CT scanner (GammaMedica, USA). The CT anatomical images were acquired using 512 projections, 50kV/600uA energy resulting in

a final resolution of $100 \times 100 \times 100 \mu\text{m}^3$. The SPECT images were acquired using an 1 mm pinhole collimator. SPECT scanning took 35-minutes (two heads, 32 projections, 60 minutes/projection) with a 20% energy window centered at 140keV, the principal energy of $^{99\text{m}}\text{Tc}$. We used a $^{99\text{m}}\text{Tc}$ -labeled radiotracer targeted at the $\alpha\text{v}\beta$ integrin ($^{99\text{m}}\text{Tc}$ -NC100692; GE Healthcare) [34,35] – a molecular marker of the angiogenic process. This radiotracer has been previously demonstrated to permit noninvasive quantification of myocardial angiogenesis.

3.1.2 Manual Pre-processing

Image Cropping: The first key preprocessing step is to eliminate the scanning bed from the CT images using a semiautomated procedure implemented within BioImage Suite (<http://www.bioimagesuite.org>). Following this, the images were cropped to eliminate portions of the mice above the ribs and regions that did not include any part of the mouse, resulting in cropped images of approximate dimensions $160 \times 90 \times 140$ (pixels), which contained the lower half of the mouse (roughly from below the lungs to the feet). We defined this graylevel microCT image as G . The same cropping procedure was repeated for the co-acquired/co-registered SPECT mouse images, we define these as S .

Image Parcellation: For both the manual analysis of the SPECT images, and for computing registration accuracy results, the mouse CT images were interactively parcellated into 7 regions, as shown in Figure 10 and previously reported in Dobrucki et al [36]. The parcellations was performed using a custom module in BioImage Suite. To summarize, planes were positioned on a 3D rendering of the CT image with the intensities adjusted to accurately visualize the skeletal structure. Two planes were used to define each hip joint, one axial above the femur and one sagittal at the pelvic joint. The knee was axially bisected between the femur and tibia/fibula. A final plane was used at the ankle joint to separate the foot. This resulted in a mouse segmentation map M , which had values 0–7, where 0 is the background and the 1–7 were used to label the following 7 regions. 1. The main thorax including the tail. 2. The left proximal hindlimb. 3. The left distal hindlimb. 4. The left foot. 5. The right proximal hindlimb. 6. The right distal hindlimb. 7. The right foot. This label map M is used in Section 3.2 to quantify local registration accuracy in terms of the overlaps of these structures post-registration. For the purposes of microSPECT quantification we masked out the bone regions from M (Figure 10) because the radiotracer is also taken up by the osteoclasts of the bone. The focus was on quantification of radiotracer uptake associated with integrin activation within vascular structures of the soft tissues. We define this bone-removed mouse map as $M^{-\text{Bone}}$.

3.2 Algorithm Validation

3.2.1 Approach—We compared our algorithm with 3 competing methods using 2 different accuracy tests, as described below. We used $N = 15$ pairs of mouse microCT images, acquired with a resolution of $100 \times 100 \times 100 \mu\text{m}^3$. These were images from the same mouse acquired at two different time points. In particular we had five baseline/3-day pairs, five baseline/7-day pairs, and five baseline/14-day pairs.

Competing Algorithms: We compared our proposed new method with 3 competing methods; an intensity-based non-rigid registration with free-form deformation (IFFD)[30,16], a robust point-based method (RPM)[11] (which forms the basis of most of the previous mouse non-rigid registration work in the literature) and the original demons registration method (which uses the same transformation model and optimization method as our method)[6].

The IFFD method was selected because it serves as a baseline, “standard” non-rigid registration method that is commonly available. It utilized a free-form deformation transformation model based on tensor b-spline and the normalized mutual information similarity metric.

RPM was selected because it forms the basis of much previous work in mouse body registration (e.g. [17,18,16]. We used our optimized RPM implementation in BioImage Suite with “two labeled” cloud sets of 5000 points from bone and skin surfaces respectively (The points cloud for bone is “inside” the points cloud for skin). RPM was run with a temperature range 1.0 : 0.2 mm the annealing factor was set to 0.9. It used a cubic b-spline tensor free form deformation model with control point spacing equal to 1.2 mm (about 12 voxels).

The final comparison was to the original demons registration method [6], which uses the same deformation model as our method – this comparison serves to illustrate the advantages of our multi-stage method.

The graylevel intensity images (at i -th day) transformed into the baseline by the registration methods are defined as $T_{proposed}(G_i)$, $T_{RPM}(G_i)$, $T_{IFFD}(G_i)$ and $T_{demons}(G_i)$.

Figure 11 shows an example of the qualitative results of the proposed registration method and other competing methods. Figure 11 (a) and (b) represents the 3 dimensional views with their coronal, sagittal and axial planes for reference and moving mice images (The images are from the same mouse at baseline and 3-days post baseline). Figure 11 (c) and (d) show their 2 dimensional views from the three planes. Lower images represent the bones (white) and soft tissues (gray) segmented from the upper CT images for results-comparison purpose. Figure 11 (e), (f), (g) and (h) show the registration results of the proposed method, IFFD, RPM and Demons, respectively. The cross-hairs in Figure 11 (c) indicates a point on the mouse’s tibia, and the cross-hairs in Figure 11 (d), (e), (f), (g) and (h) indicate the corresponding points. The registration result of the proposed method (Figure 11 (e)) shows qualitatively excellent alignment with the reference image (Figure 11 (c)) while other competing methods partially have the regions of less than satisfactory mapping in their registration results (Red arrows indicate the error regions).

Figure 12 show another example of the qualitative results of the proposed registration method and other competing methods with images from a different mouse. The registration results of the IFFD (Figure 12 (f)) and RPM (Figure 12 (g)) have good alignments for the skin boundaries. However, the alignments of bones are still unsatisfactory (Red arrows indicate the error regions of bone alignments). The registration result of the proposed method show the reliable alignments in bones as well as in skin boundary (Figure 12 (e)).

For the quantitative comparison, we performed several tests below:

Tests: 1. *Image Similarity Criteria* were used to measure image similarity following registration. We used two criteria, (i) the normalized mutual information (*NMI*) metric [31] and the correlation coefficient (*CC*) [32] as below.

$$NMI(I, J) = \frac{H(I) + H(J)}{H(I, J)} \quad (9)$$

$$CC(I, J) = \frac{cov(I, J)}{\sigma_I \sigma_J} = \frac{E((I - \mu_I)(J - \mu_J))}{\sigma_I \sigma_J} \quad (10)$$

Where I is the reference image and J is the warped target image following registration. $H(I)$ and $H(I, J)$ are the marginal and joint entropies, respectively. E and cov are the expectation and covariance, $\mu_I = E(I)$ and $\sigma_I^2 = E(I^2) - E^2(I)$.

2. *Overlap Similarity* – here we use the mouse parcellation maps M to estimate registration accuracy via segmentation. Given two binary images A and B representing a single segmented object (e.g. the left distal hindlimb) at the reference and target images respectively, we can compute a measure of registration accuracy using the Jaccard similarity coefficient (JSC) as below.

$$JSC(A, B') = \frac{|A \cap B'|}{|A \cup B'|} \times 100 \quad (11)$$

Where B' is the warped target segmentation map. $|A|$ refers the number of members in the set A as a cardinality.

3.2.2 Intensity Similarity Results—We computed the normalized mutual information (NMI) and the correlation coefficient (CC) (Table 1) for measuring the similarity of the reconstructed volume as a result of registration. They are represented as $NMI(G_{baseline}, T_{proposed}(G_i))$ and $CC(G_{baseline}, T_{proposed}(G_i))$ for the proposed registration method, and $NMI(G_{baseline}, T_{other}(G_i))$ and $CC(G_{baseline}, T_{other}(G_i))$ for the competing registration methods. In cases of the average correlation coefficient and the average normalized mutual information over the 15 mice sets, our proposed method shows superiority over all three methods (IFFD, RPM and Demons) with statistical significance (paired-T test $p < 0.05$ in Table 1). In Table 1, we calculated the first step's T test value using a paired-T test between the results of the first and third steps. Similarly, in Table 1, we calculated the second step's T test value using a paired-T test between the results of the second and third steps. In case of IFFD, RPM and demons, T tests were performed with the result of the third step. The individual comparisons over the 15 datasets are shown in Figure 13. Figure 14 displays the improvements of image registration performances in each step of our proposed serial registration.

3.2.3 Overlap Similarity Results—Table 2 shows the comparison results with the Jaccard Similarity Coefficient (JSC) for the 6 mouse regions. They are represented as:

- $JSC(M_{baseline}, T_{proposed}(M_i))$ - for the proposed registration method, and
- $JSC(M_{baseline}, T_{other}(M_i))$ - for the competing registration methods.

In the first four sub-tables in Table 2, the average JSC s of our proposed method (Third step) are slightly better or similar (Right distal hindlimb) to the average JSC s of IFFD, and always better than those of the Demons algorithm and RPM. In cases of the JSC s for both the right foot and left foot, however, our proposed serial registration performs much better than all methods (much less than 0.05 in paired-T test). We note that our method achieves consistently high overlaps over all 6 mouse regions and the improvement is particularly noticeable in the most challenging part – the feet. This is probably due to two reasons. The intensity based only methods (IFFD, demons) can not converge in these region due to the large displacements that can be present. While the imaging technologists attempt to place the mice in roughly the same orientation (the scans are acquired in weekly intervals) – this is almost impossible in the case of the feet resulting in large displacements which are less easily estimated by intensity-based methods. In the case of RPM, the improvement is most likely caused by the fact that the number of points that can be used for RPM is finite (we used about 5000 points) as it requires the computation of a match matrix which even if sparse, still becomes computationally intractable. This results in less accurate surface representation of smaller structures which explains partially why our method becomes increasingly superior to RPM as we move down the limb. (Please note that the surface only part of our algorithm – Second Step – also gives superior results to RPM, a surface-only technique, which is a more fair comparison.)

In Table 2, the right and left regions of the mouse's lower limbs show different *JSC* average values. The main reason is that the left legs were injured by femoral ligation (after baseline scanning) while right legs were used as control comparisons. Thus, the *JSC* average values of the right legs tend to be higher than those of the left legs.

Among the 15 pairs of mouse microCT images, Figure 15 shows the change of the three similarity measures of one example dataset and their corresponding images in each step of the proposed method. Normalized Mutual Information (*NMI*) and Correlation Coefficient (*CC*) were used for comparing graylevel intensity images, and Jaccard Similarity Coefficient (*JSC*) was measured for comparing bone and skin shapes in each step. At the completion of the full algorithm we can obtain *NMI* and *CC* of up to 1.536 and 0.990, respectively. As one can notice in the change of the bone and skin results, the bone similarity (JSC_{bone}) after skin registration is lower than that after the bone registration. The reason is that the displacements for the bone region are affected by the displacements for the skin region little by little during every iteration through the Gaussian kernel which is used for regularization purpose. The bone similarity (JSC_{bone}) is slightly increased by the third step of intensity registration with the increase of the skin similarity (JSC_{skin}).

Additionally, we note that we measured the final position errors by integrating over the surfaces of the reference bone and skin (using the distance map) and dividing by the number of each surface points. Our proposed method has errors of 0.35 ± 0.16 voxels for bone, of 0.64 ± 0.32 voxels for skin, and of 0.55 ± 0.25 voxels for both bone and skin surfaces for the 15 pairs of mouse images.

3.3 Algorithm Evaluation

The ultimate goal of this work is to automatically quantify the microSPECT component of the serially hybrid microSPECT/CT images. Currently, the quantification of the microSPECT component is done by manual region-of-interest (ROI) definitions performed separately in each image pair. Thus, we intend to utilize our method to automatically propagate the ROIs from the template to each imaging time point in a temporal series of hybrid images.

We present results of the application of our method to $N = 5$ pairs of mouse microCT/SPECT images acquired at different times (baseline, 3, 7, and 14 days) after femoral artery ligation of left legs (right legs were used as control comparisons). After registering the microCT images at 3, 7 and 14 days into the baseline microCT images using the proposed method and 3 competing methods respectively, then the ^{99m}Tc -labeled integrin targeted tracer was quantified from co-registered microSPECT images. The microSPECT images transformed into the baseline are defined as $T_{proposed}(S)$, $T_{RPM}(S)$, $T_{IFFD}(S)$ and $T_{Demons}(S)$, respectively. The SPECT quantification by the manual ROI definition (at i -th day) can be represented as

$ROI(S_i, M_i^{-Bone})$ while the automatic quantification of the radiotracer through the propagation of the ROI can be represented as $ROI(T(S_i), M_{baseline}^{-Bone})$. If we have

$ROI(S_i, M_i^{-Bone}) \approx ROI(T(S_i), M_{baseline}^{-Bone})$, then we can use our registration method for automatic SPECT quantification. Hence, an error of SPECT quantification is defined as

$$|ROI(S_i, M_i^{-Bone}) - ROI(T(S_i), M_{baseline}^{-Bone})|.$$

Table 3 shows the average error of radiotracer (^{99m}Tc) quantification in SPECT images between manual and automated methods for 6 local parts of mouse legs. For reference, the average quantification of radiotracer uptake using the manual method [36] over all times (baseline, 3, 7, and 14 days) for 5 mouse images were 54.22 ± 37.76 CPM (Left proximal hindlimb), 25.93 ± 15.34 CPM (Left distal hindlimb), 10.37 ± 8.74 CPM (Left foot), 41.08 ± 29.34 CPM (Right proximal hindlimb), 11.12 ± 4.96 CPM (Right distal hindlimb) and 10.95 ± 6.56 CPM (Right foot). The overall average errors in both the left proximal and the right proximal

hindlimb are larger than other leg parts because the ^{99m}Tc -labeled integrin targeted tracer tends to be heavily concentrated around the bladder (the small mis-registration of the proximal hindlimbs results in the inclusion of some parts of the main thorax near the bladder). However, the average errors of our proposed method are always better than those of other, existing methods. Figure 16 shows scatter plots of the radiotracer (^{99m}Tc) quantification error between manual and automated methods for the distal hindlimbs at 3, 7 and 14 days. Because the left and right distal hindlimbs are most important for observation of arteriogenesis/angiogenesis, only the distal hindlimbs were shown in the scatter plots (although, other parts of the hindlimbs have similar patterns of scatter plots). One can notice that our proposed method has consistently strong positive correlations in both right and left proximal hindlimbs over all times. R^2 values of the linear trendlines are also consistently higher than other methods except one case (RPM for left distal hindlimb at day 7), which means that our proposed method has more stable performance than other methods.

4 Discussion & Conclusions

We presented a new non-rigid registration method for quantitative evaluation of serial mouse images. Our method, which is based on an extended demons formulation, first aligns the bone surfaces to achieve a good initial condition and then leverages additional information obtained from skin surfaces to improve the result and finally includes the use of direct intensity information constrained by the pre-computed surface-derived transformations maps.

Unfortunately, we were not able to compare the proposed method to a similar serial registration method from Li *et al.* [18]. Li *et al.* uses two serial registration steps. The first step is a bone registration using RPM and the second step is a intensity-based registration. However, we do make comparisons to Li *et al.*'s first step of RPM based bone registration which is responsible for the majority of the displacements. The proposed method's surface based registration (step 2) performs better than the RPM control algorithm. Furthermore, the intensity based registration step in both the proposed algorithm and the Li *et al.* algorithm is mainly a "finishing step". It is not responsible for large displacements and, for the proposed method, offers only small improvements. Given this and the improvement of propose second step over RPM, we would expect the propose method to perform better than the algorithm presented by Li *et al.*. Methodologically there are similarities between the work of Li *et al.*, this work and also our previous work [27], where in each case intensity registration and surface registration were combined. However, we optimized and validated here a method that yields accurate results (both anatomically and in terms of SPECT quantification accuracy) in the very challenging region of the hindlimbs.

We further note, that the use of a distance map based representation here has two important advantages over RPM. The first is that the number of points that one can use in RPM is limited by the size of the match matrix (of size N^2 , where N is the number of points). While sparse matrix techniques can be used to reduce this, it is still limiting when dealing with whole mouse data. Second, RPM is limited by ignoring the local topology (see Figure 17) which can cause problems in narrow structures (especially if the number of points is limited), whereas the distance map representation does not have such issues.

Our proposed method demonstrates improved registration of mouse hindlimb structures than three competitor methods. In particular, as detailed in Table 2, our proposed method achieves it greatest improvement over the competing algorithms in the feet of the mouse. This result suggests that the proposed method is better than the control algorithms as the registration problem becomes more difficult, which is the case in the feet. We note that the position error (0.35 ± 0.16 voxels for bone) described in section 3.2.3 is fairly small with respect to the position error (1.75 ± 0.12 voxels for bone) as reported in [42].

When the transformation was applied to the analysis SPECT data, our method performed significantly better than the other algorithms. The scatter plots of the SPECT radiotracer at day 14 (Figure 16 (e) and (f)) show that the proposed method is significantly more correlated with the manual gold standard data. The high R^2 value for the proposed method suggests better registration of the bones. Since the specific SPECT tracer that we used, accumulates at higher concentrations in the bones, mis-registration between the soft tissues and bones will lead to the bone regions being included in the ROIs and increased CPM calculations. Table 3 shows SPECT quantification from the soft tissues and a similar result. The proposed method has significantly less error in the distal hind limbs. This illustrates that the bone had less contribution to the CPM suggesting better bone registration.

Currently, the quantification of the microSPECT component of the serially hybrid microSPECT/CT images is done by manual region-of-interest (ROI) definitions performed separately in each image [36]. Our goal is to automatically propagate the ROIs from the template to each imaging time point. From the results of this test, the proposed method showed excellent and consistent registration performances for radiotracer quantification. This allows us both to alleviate the need for this tedious manual segmentation procedure and to increase the throughput and reproducibility of data processing of multiple hybrid microSPECT/CT studies and would be applicable to other hybrid imaging systems. In addition, this method may allow the use of smaller ROIs (e.g. parcellation of the mouse limbs into muscle groups) which need only be defined at a single time point and propagated to the other time points via registration as opposed to having to manually define these subregions in each image.

Research Highlights

- Goal: Registration of articulated legs having large displacements.
- Challenge: Large displacements located close to joints in the body, which creates convergence problems.
- Methods: Serial registration method which reflects the natural physical moving combination of mouse anatomy (Bone/Skin/Soft tissue).
- Results: The proposed method shows better registration results in both the similarity comparison and the radiotracer quantification over FFD, Demons and RPM.

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References

1. Nikol S, Huehns TY. Preclinical and clinical experience in vascular gene therapy: advantages over conservative/standard therapy. *Journal of Invasive Cardiology* 2001 April;vol. 13(No. 4):333–338. [PubMed: 11287726]
2. Francis SC, Raizada MK, Mangi AA, Melo LG, Dzau VJ, Vale PR, Isner JM, Losordo DW, Chao J, Katovich MJ, Berecek KH. Genetic Targeting for Cardiovascular Therapeutics: Are we near the summit or just beginning the climb? *Physiological Genomics* 2001 October;vol.7:79–94. [PubMed: 11773594]
3. Sinusas AJ. Imaging of angiogenesis. *Journal of nuclear cardiology* 2004;vol.11(No. 5):617–633. [PubMed: 15472646]
4. Dobrucki, LW.; Sinusas, AJ. *Cardiac PET and PET/CT Imaging*. Springer; 2007. Ch 26. Imaging of angiogenesis; p. 394–411.

5. Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE. Peripheral Arterial Disease in the Elderly. *Arteriosclerosis, Thrombosis, and Vascular Biology* 1998;vol.18:185–192.
6. Thirion JP. Image matching as a diffusion process: an analogy with Maxwell's demons. *Medical Image Analysis* 1998;vol. 2(no. 3):243–260. [PubMed: 9873902]
7. Thirion, JP. Technical Report 2547, INRIA, Sophia Antipolis. France: 1995. Fast non-rigid matching of 3D medical images'.
8. Suh, JW.; Wyatt, CL. Deformable Registration of Prone and Supine Colons for CT Colonography; 2006 Proceedings of the 28th Annual EMBS International Conference; 2006. p. 1997-2000.
9. Lüthi, M.; Albrecht, T.; Vetter, T. Curvature Guided Surface Registration using Level Sets; Proceedings of the CARS 2007, 21st Congress on Computer Assisted Radiology and Surgery; 2007 Jun.
10. Suh JW, Wyatt CL. Deformable Registration of Supine and Prone Colons for CT Colonography. *Journal of Computer Assisted Tomography* 2009 November/December;vol.33(no. 6):902–911. [PubMed: 19940658]
11. Chui H, Rangarajan A. A new point matching algorithm for nonrigid registration. *Comput. Vis. Image Underst* 2003;89:114–141.
12. Papademetris X, P Dione D, Dobrucki LW, Staib LH, Sinusas AJ. Articulated rigid registration for serial lower-limb mouse imaging. *MICCAI 2005, LNCS 3750* 2005:919–926.
13. Wang H, Dong L, O'Daniel J, Mohan R, Garden AS, Ang KK, Kuban DA, Bonnen M, Chang JY, Cheung R. Validation of an accelerated 'demons' algorithm for deformable image registration in radiation therapy. *Physics in Medicine and Biology* 2005;vol. 50:2887–2905. [PubMed: 15930609]
14. Cachier P, Pennec X, Ayache N. Fast Non Rigid Matching by Gradient Descent: Study and Improvements of the "Demons" Algorithm. *Rapports de recherche - Institut national de recherche en informatique et en automatique*. 1999;(no. 3706) Note 25.
15. Guimond A, Roche A, Ayache N, Meunier J. Three-Dimensional Multimodal Brain Warping Using the Demons Algorithm and Adaptive Intensity Corrections. *IEEE Transactions on Medical Imaging* 2001 Jan;vol. 20(no. 1):58–69. [PubMed: 11293692]
16. Papademetris X, Jackowski AP, Schultz RT, Staib LH, Duncan JS. Integrated Intensity and Point-Feature Nonrigid Registration. *MICCAI 2004* 2004:763–770.
17. Li X, Peterson TE, Gore JC, Dawant BM. Automatic Registration of Whole Body Serial Micro CT Images with a Combination of Point-based and Intensity-based Registration Techniques. *ISBI* 2006;2006:454–457.
18. Li X, Yankeelov TE, Peterson TE, Gore JC, Dawant BM. Automatic nonrigid Registration of Whole Body CT Mice Images. *Medical Physics* 2008;vol. 35(no. 4):1507–1520. [PubMed: 18491546]
19. Little JA, Hill DLG, Hawkes DJ. Deformations incorporating rigid structures. *MMBIA* 1996:104–113.
20. Toga, AW. *Brain Warping*. San Diego: Academic Press; 1999.
21. Duncan JS, Papademetris X, Yang J, Jackowski M, Zeng X, Staib LH. Geometric Strategies for Neuro-anatomic Analysis from MRI. *Neuroimage* 2004;vol. 23 Suppl 1:S34–S45. [PubMed: 15501099]
22. Corouge I, Barillot C, Hellier P, Toulouse P, Gibaud B. Non-linear local registration of functional data. *MICCAI 2001* 2001:948–956.
23. Chandrashekar R, Mohiaddin RH, Rueckert D. Analysis of 3D myocardial motion in tagged MR images using nonrigid image registration'. *IEEE Transactions on Medical Imaging* 2004;vol. 23:1245–1250. [PubMed: 15493692]
24. Arsigny V, Pennec X, Ayache N. Polyrigid and polyaffine transformations: A novel geometrical tool to deal with non-rigid deformations - Application to the registration of histological slices. *Medical Image Analysis* 2005 Dec;vol. 9:507–523. [PubMed: 15948656]
25. Paigen KA. A miracle enough: the power of mice. *Nat Med* 1995 Mar;1(3):215–220. [PubMed: 7585036]
26. Davatzikos C. Spatial transformation and registration of brain images using elastically deformable models. *Computer Vision and Image Understanding* 1997;vol.66:207–222. [PubMed: 11543561]
27. Papademetris X, Shkarin P, Staib LH, Behar KL. Regional Whole Body Fat Quantification in Mice. *IPMI 2005, LNCS 3565* 2005:369–380.

28. Martin-Fernandez MA, Munyoz-Moreno E, Martin M. Articulated registration: elastic registration based on a wire-model. *Proceedings of SPIE 05 2005*;vol.5747:182–191.
29. Christensen GE, Carlson B, Chao KS, Yin P, Grigsby PW, Nguyen K, Dempsey JF, Lerma FA, Bae KT, Vannier MW, Williamson JF. Image-based dose planning of intracavitary brachytherapy: registration of serial-imaging studies using deformable anatomic templates. *International Journal of Radiation Oncology, Biology, Physics Sep*;2001 vol. 51:227–243.
30. Rueckert D, Sonoda LI, Hayes C, Hill DLG, Hawkes DJ. Non-rigid registration using free-form deformations: Application to breast MR images. *IEEE Transactions on Medical Imaging 1999*;vol. 18:712–721. [PubMed: 10534053]
31. Studholme C, Hill DLG, Hawkes DJ. An overlap invariant entropy measure of 3D medical image alignment. *Pattern Recognition 1999*;vol. 32:71–86.
32. Duda, RO.; Hart, PE.; Stork, DG. *Pattern Classification*. 2nd edition. A Wiley-Interscience Publication; 2001. p. 614
33. Vercauteren T, Pennec X, Perchant A, Ayache N. Diffeomorphic demons: Efficient non-parametric image registration. *NeuroImage 2009*;vol.45:S61–S72. [PubMed: 19041946]
34. Bach-Gansmo T, Danielsson R, Saracco A, et al. Integrin receptor imaging of breast cancer: a proof-of-concept study to evaluate 99mTc-NC100692. *J Nucl Med 2006 Sep*;vol. 47(9):1434–1439. [PubMed: 16954550]
35. Edwards D, Jones P, Haramis H, et al. 99mTc-NC100692—a tracer for imaging vitronectin receptors associated with angiogenesis: a preclinical investigation. *Nucl Med Biol 2008 Apr*;vol. 35(3):365–375. [PubMed: 18355693]
36. Dobrucki LW, Dione DP, Kalinowski L, Dione D, Mendizabal M, Yu J, Papademetris X, Sessa WC, Sinusas AJ. Serial Noninvasive Targeted Imaging of Peripheral Angiogenesis: Validation and Application of a Semiautomated Quantitative Approach. *J. Nucl. Med 2009 Aug*;vol 50:1356–1363. [PubMed: 19617325]
37. Leung KK, Holden M, Saeed N, Brooks KJ, Buckton JB, Williams AA, Campbell SP, Changani K, Reid DG, Zhao Y, Wilde M, Rueckert D, Hajnal JV, Hill DLG. Automatic quantification of changes in bone in serial MR images of joints. *IEEE Transactions on Medical Imaging 2006*;vol. 25:1617–1626. [PubMed: 17167996]
38. Maheswaran S, Barjat H, Bate ST, Aljabar P, Hill DLG, Tilling L, Upton N, James MF, Hajnal JV, Rueckert D. Analysis of serial magnetic resonance images of mouse brains using image registration. *NeuroImage 2009*;vol.44(3):692–700. [PubMed: 19015039]
39. Kovacevic N, Hamarneh G, Henkelman M. Anatomically guided registration of whole body mouse MR images. *MICCAI 2003 2003*:870–877.
40. Baiker M, Milles J, Vossepel AM, Que I, Kaijzel EL, Lowik CWGM, Reiber JHC, Dijkstra J, Lelieveldt BPF. Fully automated whole-body registration in mice using an articulated skeleton atlas. *IEEE ISBI 2007 2007*:728–731.
41. Chaudhari J, Joshi AA, Darvas F, Leahy RM. A method for atlas-based volumetric registration with surface constraints for optical bioluminescence tomography in small animal imaging. *SPIE Med Imaging 2007 vol. 6510(2007)*:651024-1–651024-10.
42. Baiker M, Milles J, Dijkstra J, Henning TD, Weber AW, Que I, Kaijzel EL, Löwik CWGM, Reiber JHC, Lelieveldt BPF. Atlas-based whole-body segmentation of mice from low-contrast Micro-CT data. *Medical Image Analysis*. 2010 In press.
43. Martín-Fernández MÁ, Cárdenes R, Muñoz-Moreno E, de Luis-García R, Martín-Fernández M, Alberola-López C. Automated articulated registration of hand radiograph. *Image vis comput 2009 July*;vol. 27:1207–1222.
44. Joshi A, Chaudhar AJ, Changqing Li, Shattuck DW, Dutta J, Leahy RM, Toga AW. Posture matching and elastic registration of a mouse atlas to surface topography range data. *IEEE ISBI 2009 2009*:366–369.
45. Wildeman M, Baiker M, Reinders MJT, Lowik CWGM, Reiber JHC, Lelieveldt BPF. 2D/3D Registration of Micro-CT data to Multi-view Photographs based on a 3D Distance Map. *IEEE ISBI 2009 2009*:987–990.

46. Xiao D, Bourgeat P, Fripp J, Tamayo OA, Gregoire MC, Salvado O. Non-rigid registration of small animal skeletons from micro-CT using 3D shape context. *SPIE Med Imaging 2009* 2009;vol. 7259:72591G-1–72591G-8.

Figure 1.

Different motions by bone and skin. (a) Bone and skin surfaces in reference image, (b) Bone and skin surfaces in moving image, (c) Displacement between bone surfaces, (d) Movement between skin surfaces.

Figure 2.
Block diagram for serial registration method.

Figure 3.

Extended demons registration method. (a) Initial direction of demons' force can be decided by the expanded surfaces (level-set) of the objects (b) Expected correct motion vectors, (c) Incorrect motion vectors by the extended demons algorithm.

Figure 4.
Detailed diagram for serial registration method.

Figure 5.

Reference and moving images and their registered results after each step during the serial registrations. The final result (f) of the serial registration looks very similar to the intensity image (top of (a)) of the reference image. (a) Reference mouse image and its bone/skin surfaces, (b) Moving mouse image and its bone/skin surfaces, (c) Wireframe rendering of the difference between the reference and the moving bone/skin surfaces, (d) Deformation results by bone surface registration, (e) Deformation results by skin surface registration, (f) Deformation results by intensity registration.

Figure 6. Level-set represented bone images generated by a distance map from the binary reference bone surface and the binary moving bone surface (Distance unit: voxel). (a) Reference bone image, (b) Moving bone image.

Figure 7. Level-set represented skin images generated by distance map from the binary reference skin surface and the binary moving skin surface (Distance unit: voxel). (a) Reference skin image, (b) Moving skin image.

Figure 8.
Spatial combination of the two displacements by distance maps.

(a) The posture of reference mouse image (Baseline).

(b) The posture of moving mouse image (Day 7) from the same mouse in (a).

(c) The posture of moving mouse image (Day 14) from the same mouse in (a).

Figure 9.

The posture of the same mouse at different scanning times. Even though a technologist attempted to position the mouse approximately in the same posture as in the first acquisition, the relative posture and their corresponding alignment of the bones vary substantially from image to image.

Figure 10.

Mouse map pairs segmented into 7 regions; main thorax including the tail (red), the left proximal hindlimb (yellow), the left distal hindlimb (light green), the left foot (green), the right proximal hindlimb (light teal), the right distal hindlimb (light blue), and the right foot (blue) (a) Baseline volume, (b) Moving volume.

Figure 11.

Registration result views by the proposed method and competing methods from moving image (d). (The Brightness of CT images were adjusted to represent the bones distinctively.). Arrows indicate the error regions being different from the corresponding part of the reference image (c).

Figure 12.

Another registration result views by the proposed method and competing methods from moving image (d). Arrows indicate the error regions being different from the corresponding part of the reference image (c). In this sample, the some of the bone registration results are inaccurate in other competing methods.

Figure 13. Similarity comparison (among proposed serial registration, IFFD, RPM and Demons) of reconstructed volumes for 15 mice datasets (a) Correlation coefficient, (b) Normalized mutual information.

(a)

(b)

Figure 14. Similarity enhancement by each step of our proposed serial registration for reconstructed volumes of 15 mice datasets (a) Correlation coefficient, (b) Normalized mutual information.

Figure 15.

An example of the variation of three similarity measures (Normalized Mutual Information and Correlation Coefficient for comparing graylevel intensity images, Jaccard Similarity Coefficient for comparing bone and skin shapes) in each step. (a) Reference image, (b) Moving image, (c) Results after bone surface registration, (d) Results after skin surface registration, and (e) Final results after intensity registration.

(a) Left distal hindlimb at day 3

(b) Right distal hindlimb at day 3

(c) Left distal hindlimb at day 7

(d) Right distal hindlimb at day 7

(e) Left distal hindlimb at day 14

(f) Right distal hindlimb at day 14

Figure 16.

Scatter plots of radiotracer (^{99m}Tc) quantification error in SPECT images between manual and automated methods for distal hindlimbs at 3, 7 and 14 days.

Figure 17.

Issues with RPM and narrow structures. RPM does not take account of the topology of the points, simply their locations. Consider the figure (a) and in particular a point on the reference surface (not shown, but of very similar geometry/topology) whose best match is the yellow point on the target surface. In this case, the yellow points has three closest “Euclidean” neighbors marked as either green (good) and orange (bad) respectively. The green points are actual neighbors on the surface whereas the orange point while physically close should not be counted as a neighbor because it is not topologically connected to the green point. In computing the match matrix for RPM, however, such points get high weight and affect the registration. The problem becomes worse if the number of points uses is too “low” to adequately represent the surface (figure (b)), as is the case in large surfaces. In this case, the orange point is much closer to the green point and would be even more disproportionately represented, and in many cases the actual matching point may end up on the “wrong” side of the hindlimb. This actually would be worse potentially in ICP-like mapping methods. While such issues are not critical for “blob”-like structures they become more critical in narrow “tubular” structures such as the hindlimbs (and especially the bone surfaces). The more dense representation available in a distance-map does not suffer from these issues.

Table 1

Post-registration Intensity Similarity Comparison for 15 mice datasets.

	CC Avg.	Std.	T test	NMI Avg.	Std.	T test
First step	0.909	0.031	2.48E-08	1.319	0.034	7.56E-14
Second step	0.969	0.010	2.17E-06	1.431	0.020	1.31E-09
Third step (Final registration)	0.982	0.010		1.496	0.003	
IFFD	0.932	0.027	2.00E-07	1.390	0.036	4.53E-11
RPM	0.937	0.023	1.42E-07	1.379	0.027	2.64E-11
Demons	0.883	0.056	3.98E-06	1.396	0.062	1.71E-06

Table 2

Similarity comparisons (Jaccard Similarity Coefficient) for the 6 regions (unit: Percent).

Left proximal	JSC Averg.	T test	Right proximal	JSC Averg.	T test
First step	65.31	3.9E-06	First step	67.66	1.65E-04
Second step	75.135	0.44	Second step	78.36	0.66
Third step (Final reg)	75.70		Third step (Final reg)	78.92	
IFFD	72.56	6.65E-05	IFFD	76.50	0.30
RPM	74.26	0.29	RPM	73.14	0.02
Demons	67.71	1.53E-02	Demons	64.67	1.10E-02

Left distal	JSC Averg.	T test	Right distal	JSC Averg.	T test
First step	62.40	9.76E-07	First step	63.44	2.03E-05
Second step	79.45	0.31	Second step	82.60	0.19
Third step (Final reg)	80.42		Third step (Final reg)	85.26	
IFFD	76.79	5.03E-02	IFFD	85.46	0.85
RPM	70.78	0.029	RPM	79.49	9.08E-04
Demons	59.52	1.70E-03	Demons	53.96	1.73E-03

Left foot	JSC Averg.	T test	Right foot	JSC Averg.	T test
First step	64.92	5.21E-08	First step	60.53	4.61E-04
Second step	78.72	0.40	Second step	79.03	0.33
Third step (Final reg)	77.50		Third step (Final reg)	83.29	
IFFD	55.36	7.54E-06	IFFD	60.51	3.09E-04
RPM	47.81	1.45E-06	RPM	58.77	6.53E-08
Demons	19.57	2.13E-06	Demons	34.34	8.39E-05

Table 3

Average error of radiotracer (^{99m}Tc) quantification in SPECT images between manual and automated methods for local parts of mouse legs (unit: count per minute (*CPM*))

Left proximal hindlimb	Average error of <i>CPM</i>	Right proximal hindlimb	Average error of <i>CPM</i>
Proposed	9.22 ± 17.77	Proposed	5.34 ± 13.13
RPM	13.09 ± 24.27	RPM	6.96 ± 14.82
IFFD	13.20 ± 24.16	IFFD	7.79 ± 16.24
Demons	12.56 ± 22.60	Demons	14.10 ± 21.47

Left distal hindlimb	Average error of <i>CPM</i>	Right distal hindlimb	Average error of <i>CPM</i>
Proposed	0.96 ± 1.23	Proposed	0.53 ± 0.46
RPM	4.18 ± 3.97	RPM	2.06 ± 2.60
IFFD	4.35 ± 2.60	IFFD	3.10 ± 2.27
Demons	5.51 ± 0.67	Demons	3.24 ± 3.84

Left foot	Average error of <i>CPM</i>	Right foot	Average error of <i>CPM</i>
Proposed	3.36 ± 5.67	Proposed	1.94 ± 1.65
RPM	4.74 ± 5.49	RPM	3.20 ± 3.86
IFFD	4.29 ± 5.51	IFFD	4.71 ± 4.46
Demons	5.91 ± 6.87	Demons	5.53 ± 5.26