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Jean-Philippe Dillenseger
Christian Goetz
Amira Sayeh
Chris Healy
Isabelle Duluc
Jean-Noël Freund
André Constantinesco
Gaëlle Aubertin-Kirch
Philippe Choquet

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Jean-Philippe Dillenseger,^{a,b,c} Christian Goetz,^{a,d} Amira Sayeh,^a Chris Healy,^e Isabelle Duluc,^{c,f} Jean-Noël Freund,^{c,f} André Constantinesco,^a Gaëlle Aubertin-Kirch,^{c,g} and Philippe Choquet^{a,b,c,*}

^aHôpitaux Universitaires de Strasbourg, Imagerie Préclinique-UF6237, Pôle d'imagerie, Hôpital de Hautepierre, Strasbourg Cedex, France

^bUniversité de Strasbourg, Icube, équipe MMB, CNRS, Strasbourg, France

^cUniversité de Strasbourg, Fédération de Médecine Translationnelle de Strasbourg, Faculté de Médecine, Strasbourg, France

^dUniversitätsklinikum, Klinik für Nuklear Medizin, Freiburg, Germany

^eKing's College London, Department of Craniofacial Development and Stem Cell Biology, Guy's Hospital, London, United Kingdom

^fUniversité de Strasbourg, Inserm, France

^gUniversité de Strasbourg, Laboratoire de Neurobiologie et Pharmacologie Cardiovasculaire, Faculté de Médecine, France

Abstract. We use high-resolution μ CT data in multiple experiments to estimate the sources of error during coregistration of images acquired on separate preclinical instruments. In combination with experiments with phantoms, we completed *in vivo* imaging on mice, aimed at identifying the possible sources of registration errors, caused either by transport of the animal, movement of the animal itself, or methods of coregistration. The same imaging cell was used as a holder for phantoms and animals. For all procedures, rigid coregistration was carried out using a common landmark coregistration system, placed inside the imaging cell. We used the fiducial registration error and the target registration error to analyze the coregistration accuracy. We found that moving an imaging cell between two preclinical devices during a multimodal procedure gives an error of about 200 μ m at most. Therefore, it could not be considered a source of coregistration errors. Errors linked to spontaneous movements of the animal increased with time, to nearly 1 mm at most, excepted for body parts that were properly restrained. This work highlights the importance of animal intrinsic movements during a multiacquisition procedure and demonstrates a simple method to identify and quantify the sources of error during coregistration.

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1 Introduction

The use of combined functional and morphological tomographic imaging has become common in preclinical imaging over the past decade.¹ As in clinical imaging, computed tomography (CT) and magnetic resonance imaging (MRI) are the modalities of choice for morphological tomographic studies, while preclinical functional tomographic imaging is mainly based on single photon emission tomography (SPECT), positron emission tomography (PET), and fluorescence optical tomography.² In general, small animal imaging techniques need anesthesia for tracer or contrast injections and adequate immobilization to prevent movement during data acquisition to enable proper registration of functional and morphological images. Maintaining animal homeostasis during all procedures can be achieved through the use of dedicated imaging cells that provide anesthesia, heating, and monitoring.^{3–5} Imaging cells can be shared by different imaging systems, limiting manipulation of animals between the different devices and facilitating multimodal combinations.^{3,6,7} The side-by-side configuration^{8,9} requires a multimodal compatible imaging cell that can be easily, rigidly, and reproducibly docked on the different imaging devices. It is manually moved from one device to another with acquisitions being performed sequentially. This is in contrast to the in-line

configuration,^{10,11} where the transfer from one modality to another relies on a linear automated and controlled translation of the imaging cell and acquisitions are performed sequentially. In both cases, neither animal nor imaging cell handling between scans is required. Fully combined hybrid systems^{5,12} also exist, where the imaging cell remains stationary and the acquisitions are carried out simultaneously.

The use of side-by-side configurations with hybrid or/and single-modal systems is still common in research units (Fig. 1), and we set ours to explore the accuracy of coregistration that can be achieved with this type of system. The sources of coregistration errors may be by either intrinsic or extrinsic movement; such movements can create artifacts and quantification errors and can, thus, interfere with coregistration and postprocessing.^{3,4} However, we have not found any quantitative evaluation of these types of movements in the literature.

In general, the coregistration accuracy will depend on multiple parameters:

- i. spatial resolution differences between modalities (I) [Fig. 2(a)],
- ii. coregistration method accuracy (II)^{8,13,14} [Fig. 2(b)],
- iii. spontaneous animal movements⁴ that we call “intrinsic movements,” (IIIa) or

*Address all correspondence to: Philippe Choquet, E-mail: pchoquet@unistra.fr

Fig. 1 The two contiguous imaging systems used in this study. No interferences were noticed between the 1.5-T preclinical MRI and the SPECT/CT, set 2 m apart.

- iv. animal movements linked to the imaging cell transportation that we will call “extrinsic movements” (IIIb) [Fig. 2(c)].

In this study, we aimed at identifying the relative movement artifacts caused by the intrinsic (IIIa) and extrinsic (IIIb) movements, using a multistep protocol. To avoid the influence of spatial resolution (I), we coregistered datasets acquired using only CT. The accuracy of the registration system (II) was already evaluated using a phantom.⁶ The purpose of this paper is therefore limited to only those multimodal workflows that require the use of a bed holder that must be moved from one system to the next.

2 Materials and Methods

2.1 Systems Descriptions and Acquisition Parameters

CT acquisitions (eXplore speCZT Vision 120, GE, Waukesha)¹⁵ were obtained at 70 kV, 32 mA, and 16 ms in 1.5 min for

phantom imaging. The acquired volume was $80 \times 80 \times 80 \text{ mm}^3$, which was sufficient for studying the head–neck, thoracic, and abdominal regions of a mouse in one acquisition; the reconstructed voxel size was $100 \times 100 \times 100 \mu\text{m}^3$. In some experiments, MRI acquisitions were done between CT scans (Table 1) because MRI sequences need considerable length of scanning time and generate acoustic noise during image acquisition, which could disturb animals and therefore stimulate motion. For MRI acquisitions, we worked with a 1.5-T preclinical MRI cryogen-free superconducting system (OPTImouse, RS2D, Mundolsheim, France). Two acquisitions were carried out successively, one T1-weighted three-dimensional (3-D)-spoiled-gradient-echo sequence (17 min) and one T2-weighted two-dimensional-fast-spin-echo sequence (12 min). The SPECT/CT system is outside the 5-G line of the MRI system.

2.2 Multimodal Coregistration System

A common landmark coregistration system consisting of a catheter fixed on a flexible plastic sheet placed between the imaging cell bed and the animal (mouse) was used.⁸ As only CT was involved in coregistration, the system was filled with iodine solution. Fiducial markers ($n = 8$) were positioned manually on the system [Fig. 4(b)] using Amide software V.1.0.5.^{16,17}

2.3 Animal Handling

The experimental protocol complied with all applicable animal welfare guidelines, including the regulations on animal experimentation (directive 86/609/EC, amended by decrees 87-848, 2001-464, and 2005-264). All experimental procedures were approved by the Institutional Animal Care Committee and were done according to French regulations concerning small animal experimentation (authorization A67-482-20). Five C57B16 mice ($23 \pm 3 \text{ g}$) were kept anesthetized (air, isoflurane 2%) in a warmed imaging cell (Minerve, Esternay, France) that enables repeated examinations on the same animal and multimodality imaging. Multimodality imaging was achieved sequentially by gently moving the cell from the SPECT/CT to the

Fig. 2 Coregistration errors between two modalities are linked with: (a) spatial resolution differences, (b) inaccurate coregistration method, and (c) animal movements.

Table 1 Experimental protocols.

Experiment numbers	1	2	3	4	5	6
Sample	Phantom	Phantom	Mouse <i>in vivo</i>	Mouse <i>in vivo</i>	Mouse <i>in vivo</i>	Mouse <i>in vivo</i>
Protocol	CT ₁ + CT ₂	CT ₁ + MRI + CT ₂ (imaging cell was manually moved between apparatus)	CT ₁ + CT ₂	CT ₁ + MRI + CT ₂ (imaging cell was manually moved between apparatus)	7 × CT (at $t = 0, 15, 30, 45, 60, 75, 90$ min)	7 × respiratory gated CT (at $t = 0, 15, 30, 45, 60, 75, 90$ min)
Registration type (n registrations)	CT ₁ + CT ₂ ($n = 5$)	CT ₁ + CT ₂ ($n = 5$)	CT ₁ + CT ₂ ($n = 5$)	CT ₁ + CT ₂ ($n = 5$)	CT _(t=0) + CT _(t=N) ($n = 6$)	CT _(t=0) + CT _(t=N) ($n = 6$)
Time between the first and the other CT acquisitions	5 min	30 min	5 min	30 min	15, 30, 45, 60, 75, and 90 min	15, 30, 45, 60, 75, and 90 min
Target(s)	Phantom cross center		Head of the left maleus—xiphisternal joint—right elbow medial epicondyle—center of C7 vertebra body—center of T11 vertebra body			
Goals	Evaluation of the registration method accuracy	Phantom registration accuracy during a CT-MRI-CT procedure	<i>In vivo</i> TRE evaluation in a single apparatus procedure	<i>In vivo</i> TRE evaluation during a CT-MRI-CT procedure	<i>In vivo</i> TRE evaluation during a 90-min single apparatus procedure	<i>In vivo</i> TRE evaluation during a gated 90-min single apparatus procedure
Types of movements explored	/	Extrinsic movements	Intrinsic movements	Extrinsic and intrinsic movements	Intrinsic movements	Intrinsic movements

MRI and vice-versa; this procedure takes <20 s (Fig. 3). Each animal was monitored (Minerve, Esternay, France) during all acquisitions. Respiratory and cardiac monitoring were achieved using a pressure pad and carbon electrodes.

2.4 Experimental Protocols

To analyze the coregistration accuracy, we calculated the fiducial registration error (FRE) and the target registration error (TRE)^{18,19} [Fig. 4(a)]. The FRE is the root mean square distance between corresponding fiducial points after registration. A PVC cylinder bored with two crossing tubes (intersection angle of 54 deg) filled with iodine solution was used⁸ to create a target point [Fig. 4(b)]. The cross intersection was considered a target to evaluate the TRE (TRE_{phantom}) [Fig. 4(c)]. The TRE is the distance, measured after registration, between a reference corresponding point other than fiducial points used for coregistration.

For *in vivo* evaluations, we used five skeletal targets to evaluate coregistration accuracy, which are defined in the captions of Fig. 4(d). Amide was used for registration of acquired data, with all points being positioned manually. To evaluate the intraoperator errors for specifying the skeletal targets, we repeated the positioning of targets on one acquisition five times. Root mean square distance and standard deviation were calculated for each target.

Six experiments were done to evaluate our separated apparatus multimodal configuration. These are detailed in Table 1 and Fig. 5. A student's *t*-test (alpha level = 0.05) was used to determine if TREs from experiments 4, 5, and 6 were

significantly different from experiment 3, which was considered an *in vivo* reference.

3 Results

The intraoperator errors for specifying the skeletal targets are given in Table 2. Intraoperator errors were, for all targets, less than the edge of a voxel ($100 \times 100 \times 100 \mu\text{m}^3$).

Figure 6 presents all experimental results. Mean TRE ($\pm$ SD) results obtained in experiments 1 and 2 are $170(\pm 40)$ and $190(\pm 50) \mu\text{m}$, respectively. The student's *t*-test ($p = 0.42$) result shows that moving the imaging cell from one apparatus to another does not have a statistically significant impact on the phantom coregistration accuracy.

For *in vivo* experiments, we observe that experiment 3 gives the highest coregistration accuracy (e.g., the lowest error). Experiments requiring increased amounts of time (4, 5, and 6) give similar results and indicate that the coregistration accuracy depends on the anatomical target point position. In the head and neck region (TRE_{maleus} and TRE_{C7}), no statistical difference is found with reference experiment 3 in all cases. However, compared with reference experiment 3, the TRE of T11 vertebra target point gives statistically significant coregistration errors ($p < 0.05$) that are observed in each experiment (4, 5, and 6). Respiratory-gated acquisitions improved coregistration accuracy only on sternum and elbow target points (TRE_{sternum} and TRE_{elbow}). Figure 7 shows the coregistration results between two sequential acquisitions, #6 and #7 from experiment 6. No transformation was applied for coregistration of the two volumes in amide, as they share the same Euclidean space.

Fig. 3 After mouse anesthesia induction, (a) we used a specific imaging cell connected to the temperature control device to maintain animal's steady state. (b) Respiratory and cardiac monitoring were achieved using a pressure pad (black arrow) and carbon electrodes (white arrows). (c, d) Mouse head was maintained with a tooth bar [(b) white star] and positioned in the anesthesia mask. (e) Mouse was positioned in the imaging chamber, which could be transported and then (f) connected to the imaging system.

Figure 7 shows the perfect matching of the imaging cell. Differences could only be noticed on animal bones.

4 Discussion

4.1 Accuracy of Fiducial and Target Coregistration

Many studies have focused on the evaluation of multimodal registration methods.^{6,9,14,19} These studies mainly discuss and compare algorithms and methodological aspects of different registration methods and do not evaluate the impact of animal movements on the registration accuracy. Our study aimed at evaluating animal intrinsic (IIIa) and extrinsic (IIIb) movements during long imaging protocols (>30 min). For all phantoms and *in vivo* experiments, FREs [line 3, (Fig. 6)] were similar, which suggests that the point-based coregistration method is reproducible and generates constant errors that are less than two voxels (200 μm). Mean TRE ($\pm\text{SD}$) results obtained in experiments 1 and 2 are 170(± 40) and 190(± 50) μm , respectively. The student's *t*-test ($p = 0.42$) result shows that moving the imaging cell from one apparatus to another has no measured impact on the phantom coregistration accuracy. Moreover, the mean TRE measured is very much lower than the SPECT spatial

resolution of the instrument used (710 μm),²⁰ confirming that the SPECT spatial resolution can be considered a limiting factor [Fig. 2(a)] in terms of coregistration accuracy.

We observed that coregistration accuracy of the head-neck region ($\text{TRE}_{\text{maleus}}$ and TRE_{C7}) (Fig. 6) is constant and gives similar results as in experiments with phantoms. However, we observed that accuracy differences in T11 vertebra (TRE_{T11}) were always statistically significant ($p < 0.05$). These results indicate that coregistration errors decreased in a caudo-rostral direction. Some research teams have developed rigid positioning systems^{3,7} that permit immobilization of animal parts (head, body, and anterior and posterior limbs) in the same position. Such rigid positioning systems could be valuable for long-term acquisitions that cover the whole-body or caudal part of the individual.

We chose to place target points on tiny bone structures [Fig. 4(d)] to maximize the accuracy of target placement and to cover the *z* direction of the acquired volume.

Mechanical vibration during CT operation does not lead to movements of the imaging cell, as shown in Fig. 7. Only the animal inside the imaging cell is displaced between two consecutive acquisitions.

Fig. 4 (a) Principles of coregistration accuracy with FRE and TRE. (b) Surface rendering of the coregistration system and its fiducial markers (white dots). The system was filled with iodine solution as well as the cross inside the phantom. (c) 3-D rendering showing the phantom with the empty cross in the middle. (d) Surface rendering of a mouse skeleton with five targets (Target 1 to Target 5) and corresponding slices through the different target points on the borders. Target 1 was the head of the malleus of the left petrous bone ($TRE_{malleus}$), target 2 was xiphisternal joint ($TRE_{sternum}$), target 3 was the medial epicondyle of the right elbow (TRE_{elbow}), target 4 was the center of the body of C7 vertebra (TRE_{C7}), target 5 was the center of the body of T11 vertebra (TRE_{T11}).

Fig. 5 Experimental protocols (1 to 6) illustration.

The intraoperator error measurement shows that the operator accuracy was sufficiently high to have no significant impact on the FRE and TRE results.

4.2 Intrinsic Movements Versus Extrinsic Movements

The last two experiments (5 and 6) did not lead to increased accuracy over experiment 4. This indicates that the drop in performance between experiment 3 and experiment 4 is not correlated with the transfer of the imaging cell between two apparatus. It seems likely that the contribution of extrinsic movements (IIIb) from the transfer of the imaging cell is not higher than the intrinsic movements (IIIa) that occur independently of movement of the cell. In fact, there appears to be a decrease in accuracy related to the duration of the acquisition/protocol (experiments 4 to 6 versus experiment 3). We note that in these experiments intrinsic movements are the main source of coregistration errors and put forward the hypothesis that the probability of intrinsic movements is constant; therefore, the longer the acquisition is, the larger the effect of

intrinsic movements will be. Even if respiratory gating is sufficiently effective at reducing chest and diaphragm blurring, our last experiment results indicate that respiratory gating is not sufficient for taking into account all movements (Fig. 6, experiment 6).²¹ This confirms that intrinsic movements could be considered a limiting factor where coregistration accuracy is concerned.

4.3 Multimodal System Configurations

Much current research is focused on the development of hybrid systems, such as PET/CT, PET/MRI,^{22,23} and more recently SPECT/PET/CT/optical for multimodality imaging.²⁴ The spread of these types of multimodal preclinical imaging methods may make the movement of animals between apparatus more common.² Our study demonstrates that moving the animal cell from one apparatus to another will not impact coregistration accuracy as long as the transport is carried out carefully. One limitation of our study is that it only applies to one particular imaging cell system and may not necessarily apply to all holders that might be commercially available. We believe, however, that we have established a simple protocol and method of analysis that can readily be applied to many types of imaging systems to establish the limits of coregistration accuracy.

4.4 Comments on Coregistration Accuracy

Many research teams are involved in the evaluation of coregistration accuracy in preclinical multimodal imaging, and registration accuracy could be studied and improved by various different approaches. One hardware-based approach consists of developing fully integrated systems to acquire functional and morphological data simultaneously (e.g., PET/MRI).²⁵ The simultaneous use of two modalities does not, however, remove the likelihood of animal movements. Other approaches include improving coregistration accuracy by developing more precise rigid or nonrigid methods,²⁶ while another proposes to obtain accurate coregistered morphological information using a deformable fully articulated atlas that can be adapted to the mouse body position.^{27,28} However, these methods require similar anatomical shape between modalities, which is difficult in SPECT/CT, for instance. In any case, none of the proposed methods are focused on eliminating the original source of animal movements. The aim of this study was not to determine the most accurate coregistration method but to evaluate the evidence-based existence of intrinsic animal movements during long-time multimodal imaging procedures, while maintaining the animal under good physiological conditions. The most effective way to limit animal movements to improve coregistration accuracy is likely to lie in preparation for image acquisition. For example, anesthetic dosage could probably be optimized to the experimental population, the individual animal, and the experimental procedure.⁴ Another development might concern

Table 2 Intraoperator errors for specifying the skeletal targets.

(n = 5)	Target 1 (maleus)	Target 2 (sternum)	Target 3 (elbow)	Target 4 (C7)	Target 5 (T11)	Mean
Mean (in μm)	27	53	33	14	9	27
SD (in μm)	35	54	37	16	10	36

Experiment number	1	2	3	4	5	6
Sample	Phantom	Phantom	Mouse	Mouse	Mouse	Mouse
			<i>in vivo</i>	<i>in vivo</i>	<i>in vivo</i>	<i>in vivo</i>
Protocol	CT-CT	CT-MRI-CT	CT-CT	CT-MRI-CT	CT x 6	Respiratory gated CT x 6
mean FRE (SD)	130 (50)	120 (40)	110 (40)	110 (50)	160 (50)	140 (30)
TRE _{Phantom} (SD)	170 (40)	190 (50)	/	/	/	/
TRE _{Maleus} (SD)	/	/	150 (80)	120 (50)	160 (70)	190 (90)
TRE _{C7} (SD)	/	/	180 (70)	140 (70)	200 (70)	220 (70)
TRE _{Sternum} (SD)	/	/	190 (90)	260 (170)	500 (140)*	310 (170)
TRE _{Elbow} (SD)	/	/	190 (80)	340 (160)*	640 (280)*	210 (100)
TRE _{T11} (SD)	/	/	200 (140)	710 (480)*	560 (410)*	970 (450)*

* Significant difference (p<0.05) comparing to experiment 3

Fig. 6 FREs and TREs with standard deviation in parenthesis (all values in μm).

Fig. 7 (a) Schematic view of a transverse μCT slice through the imaging cell during experiment 6. (b) CT slice through the imaging cell of acquisition #6 of experiment 6. (c) Same CT slice through the imaging cell of acquisition #7 of experiment 6. (d) Coregistration of the two previous slices, keeping their color scales; white arrow heads indicate the location of misregistrations.

the improvement of mouse positioning and immobilization^{3,7} in commercialized imaging cells.

5 Conclusion

This study demonstrates that moving this particular imaging cell from one apparatus to another, in a side-by-side multimodal configuration, will not negatively impact coregistration accuracy. We also highlight the existence of animal intrinsic movements during a multiacquisition procedure and that such movements have to be considered a limit of coregistration accuracy, especially for long acquisition procedures.

Disclosures

The authors declare that they have no conflict of interest.

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Jean-Philippe Dillenseger is a PhD student in biophysics in the pre-clinical imaging laboratory at the University Hospital of Strasbourg, France. His research interests are phantoms developments for multimodal preclinical imaging systems evaluation and preclinical protocols optimization for quantitative approach.

Christian Goetz is Oberarzt in the Nuclear Medicine Department of the University of Freiburg, Germany. He completed his medical school and nuclear medicine speciality at the University Hospital of Strasbourg, France. He received his PhD in biophysics and medical imaging from the University of Strasbourg in 2006. His research interests include preclinical imaging and heart imaging.

Amira Sayeh is a radiology technician. She worked for 4 years in the preclinical imaging unit of the University Hospital of Strasbourg, France. Her clinical work experience is principally MRI imaging and dental radiology at the University Hospital of Strasbourg, France. Currently, she has been pursuing her master's degree in biomorphology and biomaterials at the Medical and Dental Faculties of Strasbourg, France, since September 2016.

Chris Healy received his PhD in 1993, and he has worked on a wide range of developmental biology problems, from the specification of the musculature to the formation of the skeleton and teeth. He has extensive experience with many different kinds of optical imaging systems and, in the past 10 years, has provided the KCL Dental Institute x-ray based microcomputed tomography service.

Isabelle Duluc received her PhD in cellular and molecular biology from the University of Strasbourg, France, in 1992. She is a permanent researcher at French National Institute of Medical Research (Inserm). Her work focuses on the molecular and cellular mechanisms of gut morphogenesis and homeostasis and their involvement in diseases (cancer and inflammatory diseases).

Jean-Noël Freund received his PhD in cellular and molecular biology from the University of Strasbourg, France, in 1986. He is a senior scientist at French National Institute of Medical Research (Inserm) and director of the Unit 1113 of Inserm. His main interest concerns the development, homeostasis, and diseases of the digestive and hematopoietic systems.

André Constantinesco received his PhD in physics in 1976. He has been an MD since 1977 and a nuclear medicine specialist since 1980 at the Université Louis Pasteur of Strasbourg, France. He has been a professor of biophysics and nuclear medicine since 1981. His research interest involves blood hemodynamic, biomechanics, medical instrumentation, nuclear medicine, low-field MRI, hyperpolarized xenon MRI, and molecular imaging.

Gaëlle Aubertin-Kirch completed her pharmaceutical school and radiopharmacist specialty in 2013 at the University Hospital of Strasbourg, France. She is also a PhD student in pharmacology in the Neurobiology and Cardiovascular Pharmacology Lab at the University of Strasbourg, France. Her research interests include preclinical imaging and metabolic syndrome.

Philippe Choquet received his predoctoral degree in medical imaging and signal processing in 1991 and his PhD in medical imaging from the University François Rabelais, Tours, France, in 1996. Since 2001, he has been an associate professor in the Biophysics and Nuclear Medicine Department, CHU Hautepierre, Strasbourg. His interests include low-field MRI, MRI of hyperpolarized gases, and small animal multimodality imaging.