Chapter 1

Introduction

1.1 Background

In pre-clinical research, whole-body small animal imaging is widely used for the *in vivo* visualization of functional and anatomical information to study the different biological processes that take part in cancer, neurological and cardiovascular diseases, and help with a faster development of new drugs. Mice in particular are used, not only because they are small, have a fast reproduction rate, are easy to handle and widely available, but also because they share about 97.5% of human DNA [1, 2].

With the miniaturization of different clinical imaging equipment [3] the importance of small-animal imaging continues to grow [4]. Functional information (changes in metabolism, blood flow, regional chemical composition and absorption—physiological activities) is provided by imaging modalities such as positron emission tomography (PET), single-photon emission computed tomography (SPECT) and specialized magnetic resonance imaging (MRI). Both PET and SPECT have a high sensitivity and only nanomolar concentrations of molecular probes are needed for imaging. In SPECT, the nuclear isotopes are more readily available, cheaper, and have longer half-lives as compared to PET. PET however, has a slightly higher sensitivity [5].

Structural imaging modalities like radiography, computed tomography (CT), MRI and ultrasound provide detailed depictions of anatomy. μCT nowadays combines excellent spatial resolution with fast acquisition times. It is an excellent modality for bone imaging. Ultrasound is a real-time imaging modality that allows to visualize blood flow *in vivo*, and can be used to study blood flow and cardiac function in mice. It is extremely cost-effective and non-invasive. MRI provides good spatial resolution and has excellent contrast resolution to distinguish between normal and pathological tissue.

Optical imaging modalities, such as bioluminescence imaging (BLI), Fluorescence Imaging (FLI) and near-infrared (NIR) fluorescence imaging offer a high sensitivity in visualizing molecular processes *in vivo*. Optical imaging is fast and easy to perform, and relatively inexpensive. Bioluminescence imaging is often employed for assessing therapeutic response because of its excellent sensitivity whilst fluorescence imaging facilitates tumor growth monitoring because of its quick and convenient multiple time-point image capture [5].

Different combinations of these imaging techniques with targeted molecular contrast agents, can provide a window on the molecular processes in combination with structural changes followed in time in living animals without sacrificing them. This has the potential to advance several aspects of medicine, from fundamental research to early diagnosis to drug development.

1.2 Image processing challenges and motivation

With all these advances in image acquisition, the problem is shifting from data acquisition to data processing. The organization, analysis and interpretation of this heterogeneous integrated whole-body imaging data (Figure 1.1) has become a demanding task for the following reasons:

- the postural variability of the subjects between scans. There are little to no standardized protocols for whole-body animal imaging. If a subject is imaged using different imaging modalities and protocols, during follow-up studies or cross-sectional studies, the subject is positioned in different ways and postural variations occur (head, back and front limbs, *etc.*). This greatly complicates data analysis and comparison. Although there are some multimodality animal holders, to date they are not widely used or generally compatible with all the different scanners, and even with the use of the holders, there are still significant differences in animal posture in longitudinal studies
- the high-throughput nature of the data. More and more small-animal imaging is used in a life-span setting for drug development, in cancer studies and developmental studies. Exploring, detecting, visualizing and quantifying those changes in a robust way has become essential to utilize the full potential of the data
- the heterogeneous image structure. Some modalities provide 2D images (BLI), other, 3D (μMRI, μCT). Some are photographs, other are tomographic modalities. Some provide full whole-body information, other only functional information on only specific organs or lesions. Adding the temporal dimension, 4D images are used in follow-up and cross-sectional studies using different animals
- animal variability. Different animals are used, specific to the biological problem at hand. Different studies can have animals with different strain, size, age, body fat percentage or population. Comparing side-by-side, different animals on a common reference plane will thus facilitate the data analysis and quantification

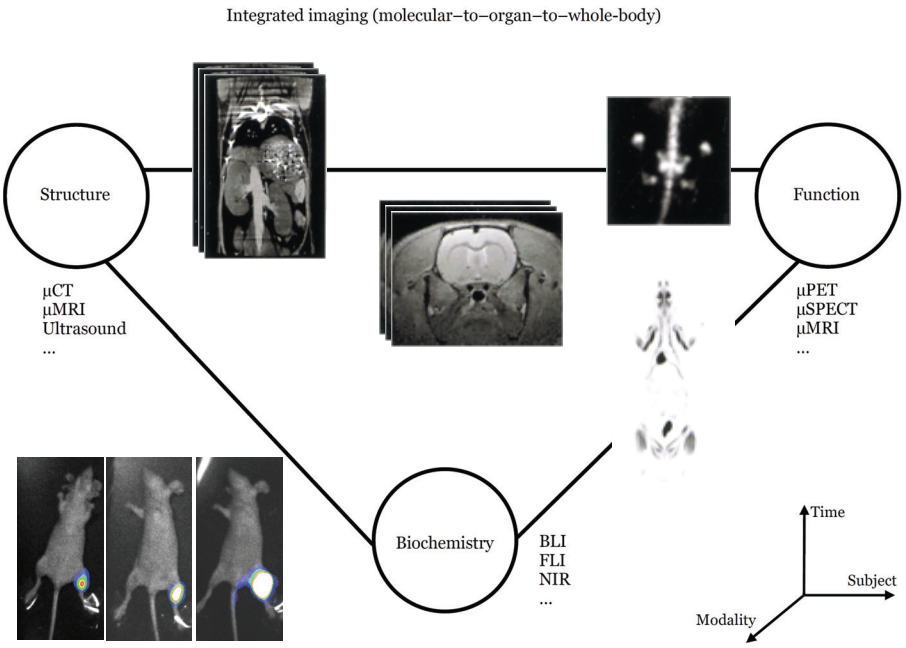

Figure 1.1 Organizing, analyzing and interpreting heterogeneous imaging data has become a demanding task for the following reasons: (i) the high-throughput nature of the data (longitudinal and developmental studies); (ii) the heterogeneous image structure (2D BLI images, 3D MRI and CT data); (iii) sometimes the whole-body is imaged, sometimes only a specific organ (*e.g.:* brain imaging); (iv) the postural variability of the subjects between scans (as depicted here in the example given for the BLI photographs)

1.3 Previous work

In recent years various approaches were proposed to deal with this multi-modal heterogeneous data in order to try to maximally exploit its information complementarity. Nevertheless, in relation to the whole-body imaging data, not much work is available. Joshi *et al.* [6] proposed a method for fitting an elastically deformable mouse atlas to surface topographic range data acquired by an optical system; Savinaud *et al.* [7] proposed a novel model-based approach to track animals in 3D from monocular video which allows the quantification of BLI signal on freely moving animals. Suh *et al.* [8] published a serial registration method to both serial µCT and µSPECT mouse images. However, these methods either do not incorporate the extremities, or were developed only for a portion of the body.

One way of handling the abovementioned problems is to use whole-body atlases. Atlases may consist of a 3D, sometimes 4D, whole-body or organ-based geometric representations. This enables mapping functional activity and anatomical variability among individuals and populations, integrating the data across modalities and quantifying change in follow-up studies. Gutierrez *et al.* [9] developed a method where automated analysis of small animal PET studies is performed through deformable registration to an atlas. This method however

depends on the availability of a correspondent co-registered CT dataset. In [10], Suh *et al.* developed a weighted demons registration method that can give preferences to particular regions of the input image using a weight image to register whole body rat CT image and PET images. Wang *et al.* [11] proposed in a simulation study the use of non-tomographic modalities like X-ray projections, to provide organ-level anatomical references of small animals in 3D by registering a digital mouse atlas. Le *et al.* [12] on the other hand developed an automatic non-rigid registration of whole body CT mice images. This method however cannot deal with very large postural differences.

As such, the recent interest in atlas based approaches has enabled combinations of different modalities, mainly in pairs. However, little to no work has been reported that enable combining several (>2) modalities into a comprehensive analysis framework, that also allows the study of follow-up data.

1.4 Contextualization

In this thesis, we further explore the approach as depicted in Figure 1.2, that served as a basis for the molecular image analysis research as performed at Laboratorium voor Klinische en Experimentele Beeldverwerking (LKEB)–LUMC. This approach is based on an *articulated* whole-body atlas as a common reference to normalize the geometric heterogeneity caused by postural differences, anatomical differences between individuals and geometric differences between imaging modalities. Mapping to this articulated atlas has the advantage that all the different imaging modalities can be (semi) automatically registered to a common anatomical reference; postural variations can be corrected, and the different animals can be scaled properly.

In the context of this framework (Figure 1.2), Wildeman *et al.* [13] proposed a 2D/3D registration of μCT data to multiview photographs based on a 3D distance map combining optical/BLI data with CT. Baiker *et al.* [14] on the other hand, presented a fully automated skeleton registration and organ approximation method using an articulated whole-body atlas in μCT mouse data [14]. This method was validated on 41 CT datasets and was successfully used to follow osteolytic lesions quantitatively and visually over time [14].

Kok *et al.* focused on integrating the whole-body data exploration across scale and time. For this purpose, he introduced the Articulated Planar Reformation (APR) algorithm [15], where after registering the articulated atlas to the data at hand, that data is reformatted along individual skeletal elements, and displayed in the atlas reference view. In this view, each volume of interest (VOI) corresponds to a single bone, which can be interactively selected for direct comparison with the corresponding VOI at another time-point or of another subject. Comparative visualization techniques that automatically highlight change over time were also provided [15].

A part of molecular image analysis research performed at LKEB–LUMC was dedicated to small animal brain data. Scheenstra *et al.* developed an automated

morphometry method for mouse brain MRI analysis with application to Alzheimer's Disease research in transgenic mice [1, 16, 17, 18].

Abdelmoula *et al.* recently proposed an automatic registration method to fuse both microscopic optical histology images and matrix assisted laser desorption ionization imaging mass spectroscopy (MALDI-IMS) data of the mouse brain. This allows to correlate the information about the neuroanatomical structures provided by the former with the chemical/molecular information provided by the latter. This assists in the early detection and diagnosis of migraine.

Mahfouz *et al.* is currently studying correlations between the specificity of certain groups of neuroreceptors to different regions of the mouse brain. The goal is to understand which genes/regulators are co-expressed with each neuroreceptor in different brain regions and link the functions of these neuroreceptors, genes and regulators to these brain regions.

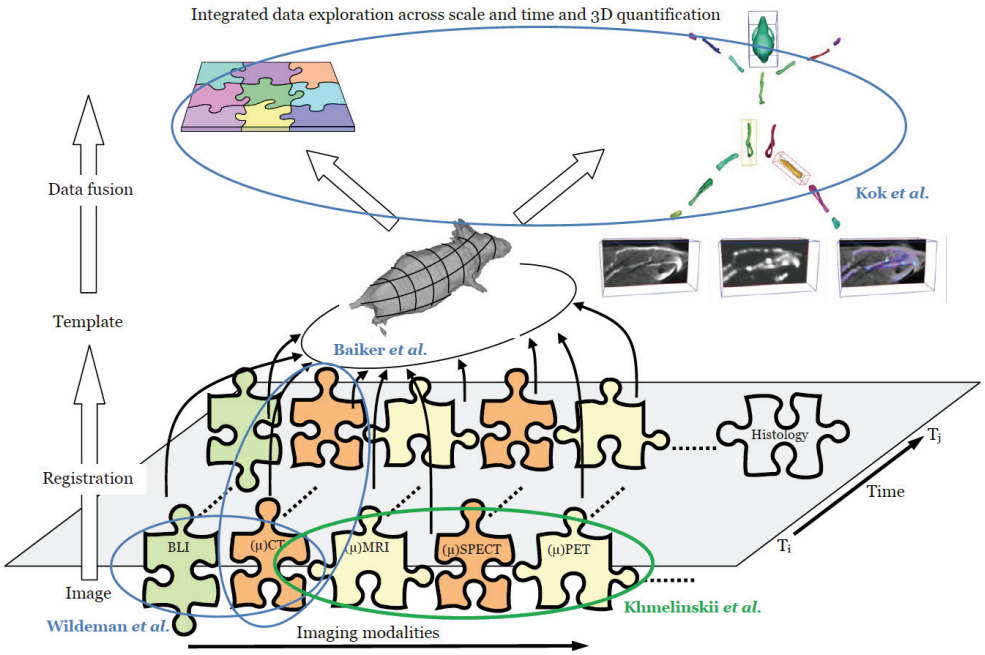

Figure 1.2 Overview of the proposed integrated approach. The data acquired using different imaging modalities for different time-points is registered to the common reference, which in this case is an *articulated* whole-body atlas. Once the data is in the atlas reference plane, it can be reformatted into individual bone VOIs. This allows normalized side-by-side visualization of the follow-up or multi-modal or cross-sectional data and quantification can be applied on a per VOI basis

1.5 Scope of this thesis

As mentioned above, in Figure 1.2, aspects such as whole-body follow-up registration and integrated visualization in the general approach have been addressed. In this thesis, we have focused on three complementary aspects, and worked towards an automated analysis pipeline for quantitative small animal image analysis. The specific goals of this thesis are:

(i) to further generalize the articulated atlas-based registration method to the multi-modality component of the global approach presented in Figure 1.2, focusing on SPECT and MRI whole-body mouse data

(ii) to expand the Articulated Planar Reformation algorithm by linking it to recently introduced resolution-enhancing MR reconstruction techniques which enable "zooming in" on small anatomical details not detectable with conventional MRI

(iii) to prove the added value of atlas-based analysis of multi-modal follow-up data in a life-science study of the ageing processes in the brain, with a specific focus on multi-contrast MR rat brain data

1.6 Thesis outline

The remainder of this thesis is structured as follows.

In **Chapter 2**, an overview of the construction process of an articulated small animal atlas together with its applications is described. Using three publicly available whole-body small animal atlases (MOBY mouse [19], Digimouse [20] and *SD* Rat [21, 22]), each skeleton is segmented manually into individual bones or bone groups. For each bone, its corresponding joint(s) locations together with anatomically realistic degrees of freedom are defined. These labeled atlases form the basis of the methodology presented in Chapters 3, 4 and 5.

In **Chapter 3,** a method for whole-body μSPECT mouse data segmentation and visual analysis is presented, provided that a tracer that is resorbed by the skeleton is used during the acquisition. It is an extension of the method previously developed for segmentation of *in vivo* whole-body μCT data combined with the articulated planar reformation (APR) algorithm. The articulated MOBY atlas presented in Chapter 2 is registered to the SPECT skeleton following a hierarchical anatomical tree. First, the atlas is coarsely registered to the entire skeleton and then, starting with the skull, each atlas bone is accurately registered to the correspondent bone in the data using the Iterative Closest Point (ICP) approach. After the atlas is registered to the data, the APR algorithm is applied to reformat the segmented data into segments corresponding to a mouse atlas and thus mapping the data to a standardized atlas space for interactive exploration and side-by-side visualization of follow-up, or multi-modal or cross-sectional whole-body data.

Chapters 4 and 5 focus on MRI mouse data. In **Chapter 4,** a novel semi-automated atlas organ and bone approximation for μMRI mouse data is presented. Guided by anatomically realistic kinematic constraints imposed by the articulated MOBY atlas the user interactively identifies a number of joints/landmarks in the MRI dataset. Individual atlas bones are automatically mapped to the target data based on the joint correspondences and the organs are mapped using Thin-Plate-Spline interpolation. In **Chapter 5,** the relevance of combining the articulated atlas-based segmentation and articulated planar reformation solutions for whole-body mouse data is further extended. An end-to-end integrated interactive approach is presented where, guided by BLI hotspots, VOIs in whole-body mouse MRI are super-resolution reconstructed (SRR) and presented in the standardized atlas space complemented by anatomical CT, for study of (micro) bone metastasis. This approach allows to overcome the limitations of CT in investigating small/micro tissue-events like micro tumors (less than half slice thickness), soft tissue tumors pathology, the homing of labeled stem cells or disease and inflammatory test pools. It also allows to overcome the high computation demand of the SRR technique when applied to large datasets (whole-body mice in this case).

Chapters 6 and 7 are dedicated to an explorative study of juvenile development and ageing processes of the brain. In **Chapter 6,** a visualization platform for side-by-side exploration of co-registered high-throughput, follow-up, cross-sectional, multi-contrast MRI rat brain data is presented. This tool is built to assist in the exploration of this high-throughput and highly heterogeneous life-span rat brain data. Its functionality and utility in molecular imaging research are evaluated by means of a case study evaluation with three domain experts. In **Chapter 7** results of this development and ageing study are presented.

In **Chapter 8,** main findings of each chapter are summarized and future work is discussed.

References

[1] Scheenstra A. *Automated morphometry of transgenic mouse brains in MR images* 2011

[2] Fox J. G., Barthold S. W., Davisson M. T. *et al. The Mouse in Biomedical Research: Diseases* Academic Press, 2nd edn. 2007.

[3] Massoud T. F. and Gambhir S. S. *Molecular imaging in living subjects: seeing fundamental biological processes in a new light* Genes and Development 17(5): 545–580 2003

[4] Lelieveldt B. P. F., Botha C. P., Kaijzel E. L. *et al. Towards integrated analysis of longitudinal whole-body small animal imaging studies* Proc IEEE Intal Conf on Acoustics, Speech and Signal Processing 5768–5771 2011

[5] Koo V., Hamilton P. W. and Williamson K. *Non-invasive in vivo imaging in small animal research* Cellular Oncology 28: 127–139 2006

[6] Joshi A. A., Chaudhari A. J., Li C. *et al. Posture matching and elastic registration of a mouse atlas to surface topography range data* Proc IEEE Intl Symp Biomed Imaging 366–269 2009

[7] Savinaud M,. de La Gorce M., Maitrejean S. *et al. Model-based multi-view fusion of cinematic flow and optical imaging* Med Image Comput Comput Assist Interv 13(Pt2): 668–675 2010

[8] Suh J. W., Scheinost D., Dione D. P. *et al. A non-rigid registration method for serial lower extremity hybrid SPECT/CT imaging* Med Image Anal 15(1): 96–111 2011

[9] Gutierrez D. F. and Zaidi H. *Automated analysis of small animal PET studies through deformable registration to an atlas* Eur J Nucl Med Mol Imaging 39(11): 1807–20 2012

[10] Suh J. W., Kwon O. K., Scheinost D. *et al. CT-PET weighted image fusion for separately scanned whole body rat* Med Phys 39(1): 533–42 2012

[11] Wang H, Stout D. B. and Chatziioannou A. F. *Mouse Atlas Registration with Non-tomographic Imaging Modalities—a Pilot Study Based on Simulation* Mol Imaging Biol 14: 408–419 2012

[12] Li X., Yankeelov T. E., Peterson T. E. *et al. Automatic nonrigid registration of whole body CT mice images* Med Phys 35(4): 1507–20 2008

[13] Wildeman M. H., Baiker M., Reiber J. H. C. *et al. 2D/3D registration of micro-CT data to multi-view photographs based on a 3D distance map* Proc IEEE Intl Symp Biomed Imaging 987–990 2009

[14] Baiker M., Milles J., Dijkstra J. *et al. Atlas-based whole-body segmentation of mice from low-contrast micro-CT data* Med Image Anal 14(6): 723–737 2010

[15] Kok P., Baiker M., Hendriks E. *et al. Articulated planar reformation for change visualization in small animal imaging* IEEE T Vis Comput Gr 16(6): 1396–1404 2010

[16] Scheenstra A. E. H., Dijkstra J. and van der Weerd L. *Volumetry and other quantitative measurements to assess the rodent brain* Methods Mol Biol 771: 277–291 2011

[17] Muskulus M., Scheenstra A. E. H., Braakman N. *et al. Prospects for early detection of Alzheimer's Disease from serial MR Images in transgenic mouse models* Curr Alzheimer Res 6(6): 503–518 2009

[18] Scheenstra A. E. H., Muskulus M., Staring M. *et al.The 3D Moore-Rayleigh test for the quantitative groupwise comparison of MR brain images* Inf Process Med Imaging 21: 564–575 2009

[19] Segars W. P., Tsui B. M. W., Frey E. C. *et al. Development of a 4D digital mouse phantom for molecular imaging research* Mol Imag Biol 6(3): 149–159 2004

[20] Dogdas B., Stout D., Chatziioannou A. *et al. Digimouse: a 3D whole body mouse atlas from CT and cryosection data* Phys Med Biol 52(3): 577–587 2007

[21] Bai X., Yu L., Liu Q. *et al. A highresolution anatomical rat atlas* J Anat 209(5): 707–708 2006

[22] Wu L., Zhang G., Luo Q. *et al. An image-based rat model for Monte Carlo organ dose calculations* Med Phys 35(8): 3759–3764 2008