Chapter 8

Summary and conclusions

In pre-clinical research, whole-body small animal imaging is widely used for the *in vivo* visualization of functional and anatomical information to study cancer, neurological and cardiovascular diseases and help with a faster development of new drugs. Functional information is provided by imaging modalities such as PET, SPECT and specialized MRI. Structural imaging modalities like radiography, CT, MRI and ultrasound provide detailed depictions of anatomy. Optical imaging modalities, such as BLI and near-infrared fluorescence imaging offer a high sensitivity in visualizing molecular processes *in vivo*. The combination of these modalities enables to follow the subject(s) and molecular processes in time, in living animals.

With these advances in image acquisition, the problem has shifted from data acquisition to data processing. The organization, analysis and interpretation of this heterogeneous whole-body imaging data has become a demanding task.

In this thesis, the data processing approach depicted in Figure 1.1 was further explored. This approach is based on an *articulated* whole-body atlas as a common reference to normalize the geometric heterogeneity caused by postural and 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 while allowing for proper management of this high-throughput whole-body data.

In this thesis, we have focused on three complementary aspects of the approach described in Figure 1.1, and worked towards an automated analysis pipeline for quantitative small animal image analysis. The specific goals of this thesis were:

(i) to further generalize the articulated atlas-based registration method to the multi-modality component of the global approach presented in Figure 1.1, 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

In **Chapter 2** we introduced three realistic, articulated skeleton phantoms derived from publicly available small animal atlases. A number of application examples using the MOBY atlas for such articulated atlases were presented. Mainly, it was demonstrated that articulated atlases can be used in correcting the postural variation, in referencing optical to CT data and in organ approximation. By combining the atlas with the hierarchical anatomical model and articulated registration, whole-body skeleton registration could be performed robustly, even in the presence of large postural variations.

The presented articulated models formed the basis of the methodology presented in Chapters 3, 4 and 5 and were made publicly available.

In **Chapter 3,** we demonstrated the feasibility of the articulated atlas-based skeleton segmentation approach combined with the articulated planar reformation algorithm for whole-body mouse bone imaging using SPECT.

Quantitative evaluation was performed by calculating the Euclidian point to surface distance between the registered atlas and the correspondent CT dataset. The obtained mean distance of 2.6 ± 2.2 voxels, showed that the registration accuracy for the SPECT data is of the same order as the previously published results for μCT, 1.8 ± 0.1 voxels [1]. The large difference in the standard deviation between the μCT fittings results and the ones presented in this paper might be due to the variable nature of the SPECT data (tracer uptake and distribution, where the tracer targets the bone growth and not the entire bone and partial volume effect) versus the more robust bone contrast in CT. Due to these factors the extraction of the skeleton out of the data may result in either a partial or a much thinner or thicker skeleton than as seen in the μCT. By collecting data from several different imaging studies, one of the goals of this study was to demonstrate the robustness of the atlas-based segmentation with respect to the different whole-body SPECT scan settings. Depending on the research question, the amount of injected tracer, the pinhole size and scan time a trade-off has to be chosen between resolution and signal. However, as long as a skeleton estimation is possible the approach presented here holds.

In [1], it was demonstrated that the proposed atlas-based segmentation method is robust with respect to osteolytic bone defects. Here, it was demonstrated, that the use of the articulated mouse atlas, with defined DoFs and size restrictions for each bone, proved to be robust for "incomplete" data (*i.e.:* images where large bits of limbs are missing). It also proved to be relatively insensitive to non-relevant objects still present in the image the skeleton extraction, like kidneys, bladder, some lung and liver. The proposed approach effectively compensated for the large variations in posture that existed within the data and yielded segmentation results requiring minimal user input. These were of satisfactory quality for the ensuing mapping of the data to the standard reference and side-by-side visualization. Applying the APR algorithm to multi-modal cross-sectional data proved to be useful to provide proper referencing and visualization for an intuitive exploration and comparison of whole-body μCT, SPECT data.

In **Chapter 4,** a semi-automated atlas-based organ approximation method for μMRI mouse data was proposed. A manually extracted set of joint & bone landmarks and the automatically extracted skin were used to determine skin correspondences, which in turn were used for a Thin-Plate Spline approximation of major organs (heart, lungs, liver, spleen, stomach, kidneys). The obtained results were generally satisfactory and similar to the manual segmentations for heart, kidneys, liver, while for other organs the atlas approximations are more variable (organs with inherent shape variability such as the stomach and spleen), and errors were larger. The calculated Dice coefficients reveal “moderate” (0.41–0.6 [2]) performance for the lungs and mostly “substantial” (0.61–0.80) or “excellent” (>0.7 [3]) performance for heart, liver, and kidneys. The comparison of the calculated Dice coefficients with previously published results shows that while performing better than [4] and at a similar level as [5], the proposed method does not obtain as good results for the lungs as the method proposed in [6] (see Table 4.1). However, the example given in Figure 4.4 reveals that the proposed TPS

mapping of the mouse major organs leads to a realistic approximation and can be used by biologists for qualitative anatomical referencing.

In **Chapter 5,** we introduced local super-resolution reconstruction (SRR) in whole-body MRI and validated its feasibility for improving visualization of tumors in small animal imaging studies. We combined a number of state-of-the-art image processing techniques in the areas of articulated atlas-based segmentation of whole-body small animal data and planar reformation to create local volumes of interest for subsequent SRR reconstruction. The approach was validated in two case studies involving CT, BLI and MRI data of bone and kidney tumors in a mouse model. Using only a few low-resolution images, and a total acquisition time compatible with *in vivo* experiments, we have produced SRR MR images from which detailed information about the metastases can be inferred. This cannot be obtained from direct MR acquisition within a feasible acquisition time. We showed that local SRR MRI is an excellent complementary imaging modality in the description of tumor metastases, and provides a high-resolution alternative to conventional MRI.

An additional point to be made is the possibility to use BLI with SRR-MRI as an alternative for the CT anatomical reference, particularly in longitudinal studies where the repeated exposure to radiation in a CT scan may become a confounding factor or cause adverse effects [7].

Chapters 6 and 7 were dedicated to an explorative study of juvenile development and ageing processes of the brain. In **Chapter 6** an intuitive and easy to use, dedicated visualization and side-by-side exploration tool for heterogeneous, co-registered multi-contrast, follow-up cross-sectional MRI data was built. The deformation field, which results from the registration step, was used to automatically link the same voxel in the displayed datasets of interest. Its determinant of the Jacobian (detJac) was used for a faster and more accurate visual assessment and comparison of brain deformation between the follow-up scans. This was combined with an efficient data management scheme. We investigated the functionality and the utility of our tool in the neuroimaging research field by means of a case study evaluation with three experienced domain scientists, using longitudinal, cross-sectional multi-contrast MRI rat brain data. Based on the performed case study evaluation we concluded that the proposed tool improves the visual assessment of high-throughput data and can further assist in guiding quantitative studies.

Longitudinal studies on brain pathology and assessment of therapeutic strategies rely on a fully matured adult brain to exclude confounds of cerebral developmental changes. Thus, knowledge about onset of adulthood is indispensible for discrimination of developmental phase and adulthood. In **Chapter 7,** by combining longitudinal MRI measurements with histological tissue characterization, a temporal profile of postnatal brain maturation was derived. Different cerebral structures showed an individual timeline for postnatal development. While the striatum seemed to be fully matured by three months, the cortex was still changing until at least six months. The presented results convincingly demonstrated that brain volume is a reliable variable of having reached a steady state situation concerning organ expansion while measurement of

cortical thickness is misleading for two to three months old rats. Clear indications of ongoing developmental changes in the rat brain pointed to the time window till three months of age; in particular, persistent myelination aspects in cortex and striatum made it advisable to speak of "adult animals" only from three months of age onwards. Future investigations on disease models or lesions will have to consider this age of three months as a safe threshold to avoid ongoing developmental changes in the brain opposing the study of the patho-mechanisms of interest.

Based on the results presented in this thesis, it can be concluded that all the formulated goals have been achieved to a certain extent. However, there is quite some room for improvement and extension of this work in the future. In **Chapter 2** the strategy applied to make the atlases articulated was outlined to deal with major postural variations (involving long bones or large bone complexes); however, it can be extended to the whole skeleton to cope with smaller anatomical variations. For example, one can define a kinematic model for each vertebra of the columna vertebralis (whereas here the columna vertebralis is defined as a single bone complex) and even to some non-rigid organs.

Chapter 3 describes a segmentation approach that was developed to cope with a scenario when a combined whole-body SPECT/CT bone scan is not always desired or available. Thus, one of the limitations of the proposed approach is the fact that the skeleton should exhibit sufficient image contrast, *i.e.,* direct application of the atlas fitting to SPECT data requires tracer uptake in the skeleton. When that is not the case, this limitation can be overcome by applying the fitting directly to the provided whole-body anatomical CT scan and then propagating the CT-fitted model to the SPECT data. Furthermore, when the method is applied to SPECT directly and correspondent CT data is not available this method requires (minimal) user input during the extraction of the skeleton out of the data. When CT whole-body data is available, this kind of user input is not required anymore, as shown and extensively validated in [1]. The approach presented here can be applied to other animals, provided there is an adequate atlas.

Chapter 4 presented a first step towards an articulated atlas-based skeleton and organ mapping for MRI data, still requiring extensive user interaction to identify the joint rotation centers. Further automation of the atlas fitting process could be achieved for example by applying atlas-based non-rigid registration, using mutual information as a similarity metric.

The results presented in **Chapter 5** represent a pilot study towards interactive super-resolution reconstruction in post mortem image data. However, we have well-founded reasons to believe that our results translate to *in vivo* imaging. Especially SRR is expected to be most successful for relatively rigid structures, such as the brain, bone tissue, and tissue surrounding bone: cases in which rigid registration will yield accurate alignment of the low-resolution images.

The interactive approach to locally reconstruct VOIs presented here, allows overcoming the time and memory limitations of the SRR techniques in large VOIs. However, as shown in Table 5.1, the mean time for the best quality SRR result, *i.e.,* using 4 low-resolution images, is still in the order of minutes—91.3 s. These results are still far from the real-time target for this approach. A re-implementation of the

algorithm in a C/C++ and GPU programming environment combination might improve these results.

Apart from oncology, the presented work flow may be of value in many research areas that requires whole body examination for local ((sub-) slice-thickness sized) effects. Examples are the homing of labeled stem cells after systemic injection, or imaging of systemic inflammatory diseases.

The platform for intuitive, integrated visualization and exploration for high-throughput co-registered multi-modal, cross-sectional follow-up data presented in **Chapter 6** would strongly benefit if combined with an image registration package such as `elastix` and features that would allow drawing and performing quantification on ROIs or other user-input templates. The presented tool, will be made publicly available for download via www.lkeb.nl

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