



Universiteit
Leiden
The Netherlands

Automated analysis and visualization of preclinical whole-body microCT data

Baiker, M.

Citation

Baiker, M. (2011, November 17). *Automated analysis and visualization of preclinical whole-body microCT data*. Retrieved from <https://hdl.handle.net/1887/18101>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/18101>

Note: To cite this publication please use the final published version (if applicable).

1

General Introduction

1.1 Molecular imaging for preclinical research

MOLECULAR IMAGING (MI) comprises a new set of technologies for “non-invasive, quantitative and repetitive imaging of targeted macromolecules and biological processes in living organisms [1].” The basis of MI consists of two elements: “(i) molecular probes whose concentration and/or spectral properties are altered by the specific biological process under investigation and (ii) a means by which to monitor these probes [1].” Therefore, imaging modalities for MI can be used for characterization, quantification and visualization of biological processes at the cellular and molecular level in living organisms [2]. In contrast to imaging modalities such as anatomical Magnetic Resonance Imaging (MRI), which rely on non-specific physical or metabolic changes to distinguish pathological from physiological tissue, molecular imaging enables the identification of events like the expression of a particular gene [3]. MI therefore offers great new opportunities because molecular events, that are related to a certain disease, can be detected long before the disease manifests itself by macroscopic anatomical modifications. Because of its non-invasive nature, it also allows to monitor disease progression over time, in a physiologically realistic environment and within the same subject. Compared to classical follow-up studies, in which a part of the cohort had to be sacrificed at each time point, using the same subject over time removes intersubject variation and the studies require significantly less animals [2]. Further applications for MI range from studying gene expression and intracellular events to detection of cell trafficking patterns related to inflammatory diseases and metastases and monitoring therapeutic effects of new drugs by assessing drug distribution and effectiveness [4–6].

For MI, almost all imaging modalities commonly utilized in clinical practice are used ranging from molecular Magnetic Resonance Imaging (MRI), Computed Tomography (CT) and Ultrasound (US) to the nuclear modalities Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) and the optical modality Fluorescence Imaging (FLI). The prerequisite for imaging is that there exists a suitable molecular probe for a particular modality. Since the dimensions of the subjects

Chapter 1

in preclinical research are one order of magnitude smaller compared to patients in the clinic, scanning hardware was developed in recent years that is tailored for small animal imaging. The main advantages of preclinical scanners are their compact size, the high resolution, the possibility to scan entire subjects and that some additional modalities are available, for example Bioluminescence Imaging (BLI). Generally, preclinical scanners can be identified by the prefix ‘Micro’ (see Fig. 1.1).

MI modalities offer a very high sensitivity but generally their spatial resolution is low, which complicates localization of the signal within the animal [7]. Therefore, often datasets of multiple modalities containing complementary information are ‘fused’. An example is given in [5], where BLI is used to monitor metastatic activity of breast cancer cells in the mouse skeleton over time. In addition to the BLI signal, a photograph of the animal is acquired that enables to coarsely estimate the location of the metastases. To visually assess the cancer-induced bone resorption, the data is combined with whole-body MicroCT (Fig. 1.2). Besides visual investigation, multimodality fusion may be required to enable quantification of a molecular probe. Examples are the optical modalities BLI and FLI, which require a realistic tissue model of the studied subject based on another modality, in order to accurately determine the location and the emission of a light source within a subject [8, 9]. Another type of application where multimodality imaging is required is computer-assisted scan planning. Approaches are typically based on matching a prior model of the anatomy of interest to a set of scout views. An example is given in [10], where an anatomical mouse atlas was registered to a sparse set of scout photographs, yielding scan Volume-Of-Interest (VOI) estimates for subsequent MicroSPECT data acquisition.

1.2 Image processing challenges

Because of the non-invasive nature of MI, often several data acquisition time points are planned to follow a certain disease or treatment effect over time, within the entire animal. To be able to compare the results, datasets of different time points have to be aligned (registered) to each other. However, this is particularly difficult for whole-body data because of non-standardized acquisition protocols and the fact that the body contains many different tissues with largely varying stiffness properties. This results in a potentially large postural variation between animals that are imaged at different time points (Fig. 1.3, left and middle) or if different animals have to be compared. This postural variation is caused by articulations of the skeletal system, deformations of soft tissues and anatomical difference between animals. While many strategies are discussed in the literature [12–15] for registration of individual objects like the brain or the heart, only few methods aim at registration of objects with greatly varying structural properties and articulations.

Another problem that arises when studying whole-body data is the fact that it may be difficult to image all anatomical structures of interest at the same time with only one modality, because each modality has a specific target. While MRI for example is suitable for imaging soft tissues, it yields no bone contrast whereas *in vivo* MicroCT data shows excellent bone contrast but only poor soft tissue contrast (Fig. 1.3, right). In exceptional

General Introduction

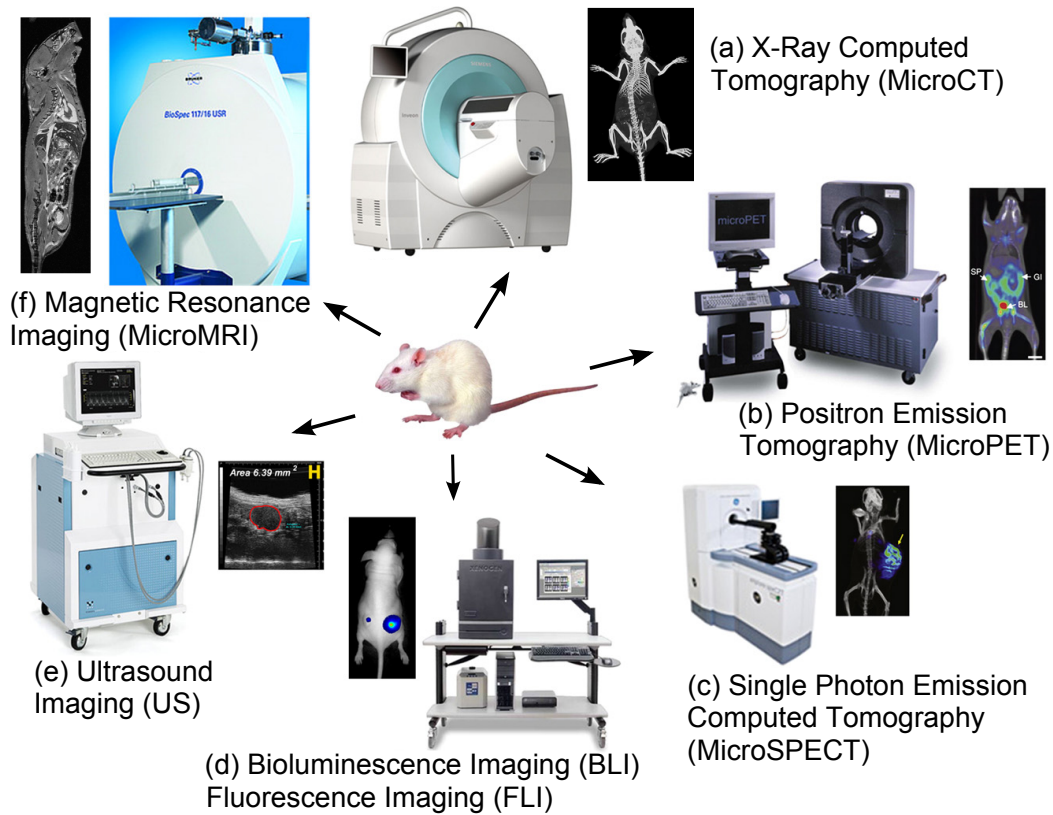


Figure 1.1: Modalities used for preclinical molecular imaging. Figure adapted from [11].

cases, contrast-enhanced MicroCT can be used to obtain soft tissue contrast. However, this solution is usually not preferred because contrast agents are difficult to administer and may influence the outcome of follow-up studies. Thus besides the combination of a functional and an anatomical modality, it sometimes may be necessary to add another anatomical modality.

The necessary combination of modalities for MI leads to another challenge for image processing because datasets from several modalities have to be brought into correspondence. Sometimes researchers can rely on hybrid acquisition hardware integrating several modalities in one setup and thus circumvent this problem. However, these solutions are often not available and not all modalities can be combined.

In conclusion, the challenges for image processing are:

- Potentially large postural variations that complicate comparing animals in follow-up (same animal, multiple timepoints) and cross-sectional (different animals, one timepoint) studies,
- Large tissue heterogeneity of whole-body data with greatly varying stiffness properties and
- Absence of geometrical calibration between scanners in multi modality imaging.

Chapter 1

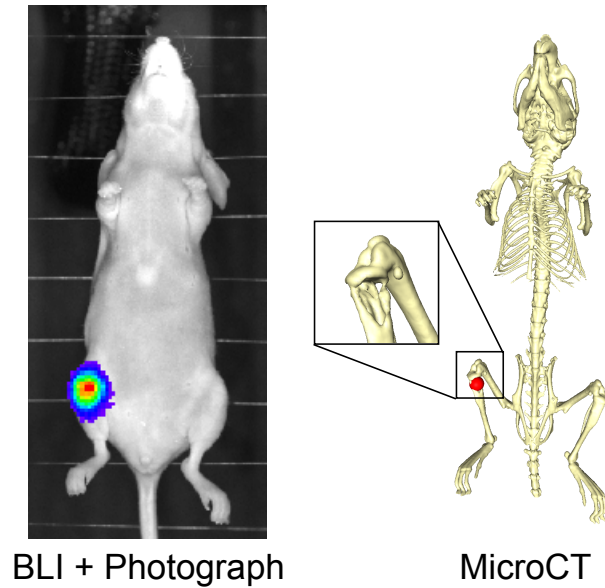


Figure 1.2: Demonstration of multimodality imaging for BLI and MicroCT. For coarse light source localization, the BLI signal is overlaid on a light photograph (left). For accurate localization or quantification of e.g. osteolysis, a MicroCT dataset should be acquired as well (right). The red sphere represents an approximation of the light source.

1.3 State of the Art

1.3.1 Registration of whole-body data

For some applications, especially designed holders can be used in order to scan animals in similar position at different time points and thus reduce postural variability [18]. However, such holders may influence the study e.g. by obstructing light in optical imaging based studies and therefore software based solutions for registration of datasets are required as well.

In the literature, several approaches are described to tackle the aforementioned difficulties of whole-body registration. This review mainly focuses on methods for small animal applications. Reported are:

1. *Methods that are based on global image data i.e without including prior knowledge about the internal structure.* Approaches are based on intensity in MicroCT [19] or on extracted features like the mouse skin [20,21].
2. *Methods that distinguish between different tissue types, based on grayvalues.* One of these approaches is presented in Staring *et al.* [22], where the authors filter the deformation field after each iteration step of the registration, dependent on the tissue rigidity. In [23], the same authors include a rigidity term in the registration criterion. In order to do so, the rigidity has to be determined based on the image data. However, since the method is based on CT data, there is a correlation between tissue rigidity and radio density and rigidity is derived from the Hounsfield units.

General Introduction

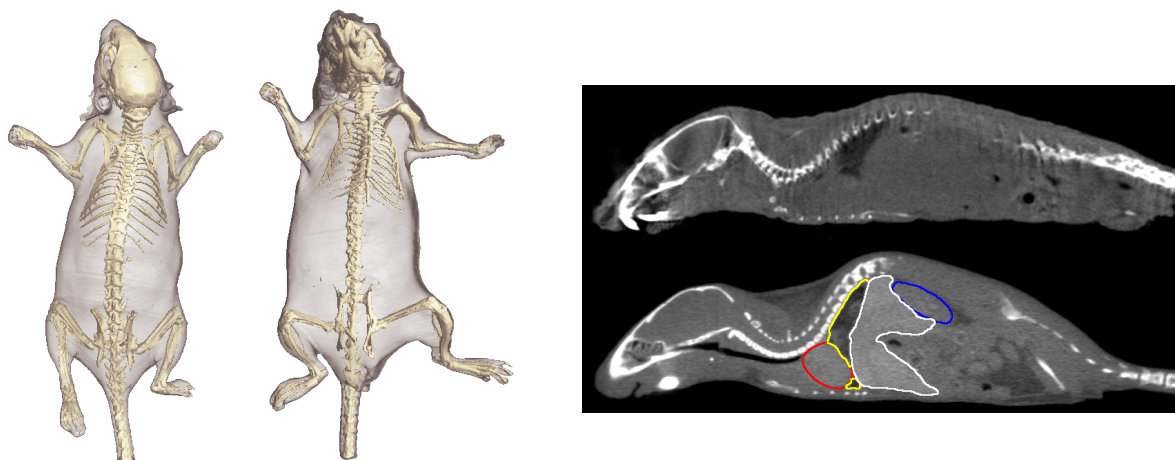


Figure 1.3: Postural variation for prone (left) and supine (middle) data acquisition. Figure adapted from [16]. Demonstration of soft tissue contrast in non-contrast-enhanced in vivo MicroCT (right, top) and contrast-enhanced in vivo MicroCT (right, bottom). The labels indicate the heart (red), the lungs (yellow), the liver (grey) and the kidneys (blue). Figure adapted from [17].

3. *Methods that distinguish between different tissue types based on an initial global segmentation.* Xiao *et al.* [24] register two surface representations of a mouse skeleton. Other methods describe a two step approach. First, only the segmented regions are registered, followed by an intensity-based registration step. In Li *et al.* [25,26] and Suh *et al.* [27], the authors first register the skeleton. In [27], the skin is registered as well, initialized by the result of the skeleton registration. In both approaches, the results of the first step are used to initialize a deformable registration of the entire body. In either case, the modality is MicroCT.
4. *Methods that are based on registration of local image data and subsequent derivation of a global transformation, so-called block-matching methods.* Although these methods in general do not include prior knowledge about the internal structure, the locality of the individual registrations can, to a certain extent, handle varying tissue properties and articulations, depending on the type of transformation. The reviewed methods are all based on intensity and methods differ in the transformation models of the individual blocks. Transformations include translation only [28], translation and rotation [29] and affine [30,31] local transformations. In all of these approaches, the blocks are registered independently. There is one exception [31], where the blocks are registered simultaneously. A block-matching approach that does include a priori knowledge by means of a hierarchical animal model is presented in Kovacevic *et al.* [18]. In their work, the authors register whole-body MRI data by first registering the entire body, subdividing the result, register again, subdivide again and so on. They identify individual bones and organs in a reference dataset and use affine transformations for registration of individual elements.

Chapter 1

5. *Methods that are based on registration of local image data and subsequent derivation of a global transformation, where the local transformations are constrained by including a priori knowledge of the anatomy of the subject.* This can be achieved by including a kinematic model of articulated structures. Martin-Fernandez *et al.* [32] use an articulated hand model and register it to 2D hand radiographs. The individual bones, represented as rods, are initialized by the result of the previous registrations and the transformation is constrained by anatomically realistic motion constraints of the hand joints. Du Bois d’Aische *et al.* [33] register a human head, based on a model of the cervical spine. Articulated vertebrae are registered to the target image and the deformation is propagated to the rest of the head using a linear elastic model. Bones are registered simultaneously, but motion between cervices is small. Van de Giessen *et al.* [34] register the bones of the wrist by imposing motion constraints to prevent unrealistic constellations. All bones are registered simultaneously, but they have to be identified in advance. Papademetris *et al.* [35] use a kinematic model to register the legs of a mouse by modeling the joints. Articulated parts have to be segmented manually.

The presented solutions in Items 1-4 vary greatly in their capability to properly treat tissue heterogeneity and to handle postural variations. The solutions in Item 1 may cause internal tissues to deform in non-realistic ways. However since in [20] and [21] only a representation of the skin is needed, an anatomical organ atlas can be registered to any modality yielding a segmentation of the data. The solutions in Item 2 and Item 3 ensure more realistic deformations for the skeleton [24], the skeleton and the skin [25–27] or various soft tissues [22, 23]. Due to the dependency on a segmentable skeleton and tissue density maps, these methods are mainly restricted to intramodality registration using CT. The methods in Item 4 are capable of handling multimodality data, because registration of the individual blocks is generally intensity-based.

Common to all methods discussed so far is that they may suffer from local minima during registration because of limited capability to handle large postural variations, or if bones lie in close proximity like e.g. around the ribcage. In these situations, obtaining the correct result cannot be guaranteed.

Most approaches in Item 5 are inherently more robust to deformations caused by articulations, because these articulations are explicitly modeled and taken into account during registration. To date, all these methods derive local transformations of target structures and if desired, determine a global transformation using a weighted combination of the local transformations [18, 32], a linear elastic model [33] or a solution to ensure global invertibility [35].

None of the approaches discussed so far did address the problem of dealing with structures that do not show sufficient contrast for registration, [20] and [21] being the only exceptions. In their work they demonstrate how registration using an anatomical animal atlas (the Digimouse [36]) can compensate for missing structural information.

General Introduction

1.3.2 Registration of datasets from different modalities

Molecular imaging applications typically rely on multimodality image acquisition to combine functional and structural data and thus to facilitate visual localization and quantification of molecular events (Sec. 1.1). Many solutions for multimodality image fusion are described in the clinically oriented literature. The most relevant application areas are oncology, cardiology, neurology as well as radiation therapy planning or assessment of therapy and typical modality combinations are MRI or CT and PET or SPECT [13,15,37].

With preclinical (animal) imaging, the variety of imaging procedures is even larger than in clinical imaging, since almost every research question to be answered requires a unique protocol. Consequently, many multimodality studies based on small animals require a specific solution to register two or more modalities. Currently, preclinical counterparts of clinical hybrid systems became available that allow to acquire PET and CT [38], SPECT and CT [39], PET and MRI [40] as well as SPECT and MRI [41] (refer to [42] and [43] for reviews). After data acquisition, the datasets can be ‘fused’ directly because all of the modalities yield 3D data, registered by hardware. The same is true for 2D hybrid systems like the combination of radiography and optical imaging in one device [44].

Other possible modalities that are used in combination differ in data dimensionality and are typically combinations of 3D data and 2D data. Douraghy *et al.* [45] present an integrated Optical-PET (OPET) scanner that is currently under development and FLI-PET [46] and CT-Optical [47] systems are also presented. A very interesting novel approach is presented in Hillman *et al.* [48] where the authors only rely on an all-optical system to derive the location and shape of all major organs. To this end they measure the biodistribution dynamics of a fluorescent dye and subsequently derive organ boundaries. At the same time, MI information is acquired.

Imaging experiments using several single modality acquisition hardware require software solutions to register the data after acquisition. Most methods are based on using the same animal holder in all modalities and animals are usually anaesthetized during scans to minimize body movement induced artifacts. Therefore most studies include a rigid transformation model. Again, handling datasets with equal dimensionality are most straightforward. Combinations of volumetric data are PET-CT [49], PET-MRI [50,51], SPECT-CT [52] and CT-PET-SPECT [53]. Image similarity measures are generally based on intensity or intensity gradients but can also be feature-based. A projection of structured light can be used to reconstruct the skin surface of an animal in 3D and subsequently registered to CT [54] and MRI data [55].

It gets more complicated if data of different dimensionalities need to be fused, because derivation of a similarity measure is not straightforward any more and the transformation model usually has to incorporate scaling parameters as well. Although it is possible to circumvent these issues and make use of external [8,10] or implanted fiducial markers [56], these solutions require significant user effort to place the markers and generally have limited accuracy. Within the image processing literature regarding small animal data, very little work has been published on fully automated registration of data with different dimensionality. Exceptions are two methods for registration of 2D projections of a mouse skin, derived from 3D MicroCT data, and one [57] or three [58] 2D photographs of the same animal including an affine and rigid transformation model respectively.

Chapter 1

1.4 Goals of the research

Based on the particular challenges that arise for imaging entire bodies (sec. 1.2), the overall goal of this thesis is to develop methods for the analysis and visualization of cross-sectional and longitudinal (follow-up) whole-body small animal imaging data.

In particular, we focus on developing methods that:

- Are highly robust to large postural variation
- Can deal with the large heterogeneity of animal bodies
- Can compensate for lacking tissue contrast and
- Can facilitate the combination of multiple modalities.

1.5 Outline of the thesis

This manuscript is organized as a collection of scientific papers: consequently, a certain degree of content overlap will be present in the most general parts of the following chapters. The context and novelty of each chapter are described here.

In Chapter 2, the process of developing an articulated animal atlas is described. Based on labeled 3D volume datasets of three publicly available whole-body animal atlases (MOBY mouse [59], Digimouse [36], SD Rat [60,61]), the skeletons are segmented manually into individual bones in a first step. Second, joint locations are defined and anatomically realistic motion constraints are added to each joint. Finally, surface representations of the individual bones and major organs (skin, brain, heart, lungs, liver, spleen, kidneys, stomach) are combined, yielding a representation of the atlas that forms the basis of the methods presented in the following chapters. In addition, some application examples for usage of such an atlas are given. The atlases are made publicly available (<http://www.lkeb.nl>).

In Chapter 3, a novel and highly robust method for segmentation of in vivo whole-body MicroCT data is presented. It is based on a combination of the articulated MOBY atlas developed in Chapter 2 and a hierarchical anatomical model of the mouse skeleton, and enables to achieve a fully automated registration of the atlas to a skeleton surface representation from the target data. First, the entire skeleton is coarsely aligned and subsequently, individual bones are registered one by one, starting with the most proximal bones and ending with the most distal bones. This renders the method highly robust to postural variations and greatly varying limb positions. Other high contrast organs, namely the lungs and the skin, are registered subsequently, initialized by the skeleton registration result. In a final step, low-contrast organs are mapped from the atlas to the target by means of Thin-Plate-Spline interpolation. The main novelty of the method is the high robustness with respect to postural variations and the usage of a whole-body atlas, to compensate for missing organ contrast. Another novelty is the robustness with respect to severe bone malformation because of e.g. metastatic activity. The Degrees of

General Introduction

Freedom of the individual bones are constrained such that even large holes in the skeleton do not cause the registration of the individual bones to fail.

Possible applications of the automated whole-body segmentation include anatomical referencing and it allows to provide a heterogeneous tissue model of the target that can be used to improve the light source reconstruction in optical tomography approaches. Another application example is the qualitative analysis of differences between individual bones or organs in intramodality follow-up or cross-sectional datasets. Chapter 4 describes, how the result of the atlas-based skeleton registration can be used for mapping multiple time points of a follow-up MicroCT datasets into a common reference frame (Articulated Planar Reformation, APR) and be visualized side-by-side. Above that, several change visualization strategies are discussed that can help researchers to easily follow e.g. a certain therapeutic effect over time without any user interaction. The novel aspects in this chapter are the framework for automated navigation through whole-body data and the side-by-side assessment of whole-body follow-up data.

In Chapter 5, the APR framework is extended by a concrete example, where accurate quantification is required to follow disease progression over time. More specifically, tibial tumors are induced by breast cancer cells and osteolysis is followed over time in whole-body MicroCT datasets. To this end, a structure of interest, in this case the tibia, is selected automatically in each time point and combined with a highly accurate segmentation strategy and subsequent measurement of bone volume changes over time. Besides that, a way to determine and visualize cortical bone thickness is demonstrated. Thorough statistical analysis reveals that the segmentation results of the automated method and two human observers do not differ significantly. The novelties presented in this chapter are the analysis of osteolysis in 3D data, the automated segmentation of a particular structure of interest in whole-body in vivo data and the automated derivation and visualization of cortical bone thickness maps.

The atlas-based skeleton registration presented in Chapter 3 proved to be highly robust to postural variation and pathological bone malformations. However, this is at the expense of bone registration accuracy. In Chapter 6, the robustness of the articulated registration is combined with the accuracy of an intensity-based registration algorithm. An intensity-based similarity criterion is regularized with the corresponding point information obtained from the articulated registration. Registration is formulated as an optimization problem and solved using a parameter-free and very fast optimization routine in a multiresolution fashion using Gaussian pyramids. It is shown that the combination of intensity and the corresponding point information outperforms methods based on either intensity or corresponding point information alone and that the method is highly time efficient, compared to other published work.

Another important aspect of preclinical MI applications is addressed in Chapter 7. The goal of the presented work is to automatically register multimodality, multidimensional data, namely 3D MicroCT of an animal and two or more 2D photographs of the same animal, taken at different viewing angles. 2D photographs are often taken together with Fluorescence and Bioluminescence data and with the automated registration, the FL and BL data can be related to 3D anatomical MicroCT data without the requirement of two calibrated systems or the knowledge of the between-system transformation matrix.

Chapter 1

The only requirement is that the animal is placed on a multimodality holder and does not move during the transport from one scanner to the other. The fact that the registration is performed in 3D, based on an approximate reconstruction of the skin surface from the 2D projections, renders the method fast and flexible; the more 2D projections are available, the better the 3D reconstruction and therefore the registration accuracy but without noticeable increase in time requirement for the 3D reconstruction. Chapter 8 summarizes the findings of this thesis and presents some areas of future work.

General Introduction

