TOWARDS INTEGRATED ANALYSIS OF LONGITUDINAL WHOLE-BODY SMALL ANIMAL IMAGING STUDIES

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ABSTRACT

This paper discusses a number of image analysis challenges emerging from longitudinal small animal molecular imaging studies. Three steps towards a quantitative 3D analysis of follow-up small animal imaging are presented: whole-body articulated registration, change visualization in follow-up data and fusion of optical and 3D structural imaging data. Several application examples are presented in the context of translational cancer research.

Index Terms— Small animal imaging, whole-body registration, change visualization, image fusion

1. INTRODUCTION

The importance of small-animal imaging in life-sciences research has grown considerably over the past decade. In part, this is caused by the rapid progress in image acquisition hardware, where many small-animal equivalents of clinical modalities have been developed. Structural imaging modalities such as μMRI, μCT, and ultrasound, provide detailed depictions of anatomy. μPET, μSPECT, and specialized μMRI protocols add functional information. In parallel, great progress has been booked towards selective visualization of biochemical processes in-vivo. For instance, optical imaging modalities, such as bioluminescence imaging and near-infrared imaging, offer an unprecedented sensitivity in following gene expression and protein interaction in action: combined with the abovementioned structural and functional imaging modalities, this enables the in-depth study of the molecular origin of a disease in relation to the structural and functional changes they cause [1].

In pre-clinical research studies, the heterogeneous imaging data is often still interpreted by qualitative visual inspection on a per-modality basis, whereas the complementarity of all data would be maximally exploited by an integrated, 3D quantitative analysis of disease processes [1]. As such, there is a great demand for analysis techniques that quantify and combine all information in follow-up small animal imaging studies, preferably with a high degree of automation. A number of registration and segmentation requirements are indeed similar to clinical imaging modalities, and have been addressed in small animals as well (e.g.[2]). However, several new data processing challenges have emerged from the specifics of small animal modalities. Examples of such challenges are:

- Whole-body image analysis in the presence of extremely large variations in posture between experiments. This is due to the fact that standardization of animal posture in the acquisition devices is challenging because of varying physical and experimental constraints. This greatly complicates coherent data analysis, both in cross-sectional and follow-up studies.
- Detecting and visualizing changes over time; small animal imaging is often used in a high-throughput setting, for instance for compound screening in pre-clinical studies. Therefore, methods for detection and quantification of changes over time are required that are highly robust with respect to the aforementioned data heterogeneity. Also, intuitive ways of visual data exploration in such large, heterogeneous follow-up studies are required.
- Geometric structure and information content of the image data is highly heterogeneous. Some modalities consist of planar animal photographs, others are tomographic 3D studies with anatomical information on the whole body (μMRI, μCT), or functional information on only specific organs or lesions (μPET, μSPECT).
- Novel signal quantification needs. Several molecular modalities (such as bioluminescence and fluorescence imaging) offer great sensitivity in detecting molecular events, however quantification of concentration and position of light-emitting sources is challenging. This requires correction methods for photon scatter and attenuation that integrate heterogeneous 3D tissue atlases.

In this paper, a number of developments from our group are described towards a system for integrated 3D quantitative image interpretation that addresses the abovementioned challenges. Application examples are given in the context of screening novel pharmaceutical compounds that inhibit metastasis formation in breast cancer [3,4]

2. METHOD OVERVIEW

Figure 1 provides a schematic overview of the proposed approach. The crucial first step towards integral, 3D image analysis is normalizing the geometric heterogeneity caused by postural differences, anatomical differences between individuals and geometric differences between imaging modalities. To address this first problem, we have developed novel registration algorithms to map the heterogeneous image data to a whole-body anatomical atlas (Section 3). This enables the subsequent steps of our analysis pipeline: an integrated follow-up analysis over time (Section 4), and across modalities (Section 5).
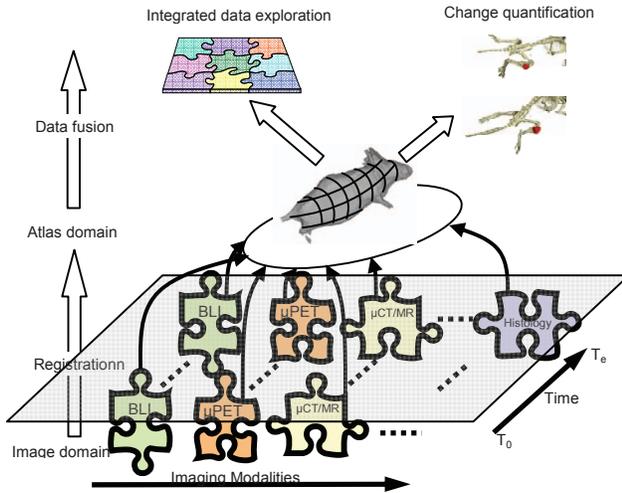
Figure 1: A key step towards an integral, 3D analysis is to normalize for geometric heterogeneity between modalities and time points by registration to a whole-body atlas. This enables a data integration across modalities and change quantification and visualization in follow-up studies.

3. WHOLE-BODY REGISTRATION

The challenge of image registration in the presence of high postural variability has recently received increasing attention ([5-12], see [12] for a detailed review of related work). We have developed an approach that uses explicit knowledge on skeletal degrees of freedom to constrain the registration process [12]. To realize this, we have divided the problem of whole-body registration into an atlas constrained registration based on high-contrast organs in μCT (skeleton, lungs and skin), and a soft tissue approximation step for low-contrast abdominal organs. Using three publicly available small-animal atlases (MOBY [13], Digimouse [14], Sprague-Dawley rat [15]), we built three articulated atlases, where major bones/bone groups were manually segmented for each atlas skeleton. Then, a kinematic model for each atlas was defined: each joint position was identified and the corresponding degrees of freedom were specified [16]. The articulated adaptations to these atlases have been made publicly available for download.

To register the atlases to target data, we employ the articulated skeleton by executing a hierarchical registration procedure from proximal to distal bone segments. The more distal a given bone in the skeleton, the more variable its position between acquisitions. Given that the entire atlas skeleton is coarsely aligned to a target dataset in a first step, all bones can subsequently be matched individually. The registration of a distal segment is constrained by the joint type of the proximal bone it connects to. For example, for the tibia, the registration is constrained by the DoFs of the knee joint. Since the registration has to deal with large deformations, pathological changes and inter-subject variations, a rigid transformation model including non-isotropic scaling was chosen. The registration was embedded in the Iterative Closest Point framework, while constraining the Degrees of Freedom of the registration depending on the joint type and motion range. This renders the method highly robust to exceptionally large postural differences between scans and to moderate pathological bone deformations.

The registered skeleton allows for an initial estimate for the registration of several other major internal organs, because their location strongly depends on the animal posture. In the absence of clear tissue contrast between abdominal organs in μCT, an organ approximation is performed through thin-plate-spline (TPS) mapping of the atlas organs to the target data. The anatomical landmarks that define the TPS mapping are primarily derived from the registered skeleton. To this end, we compute a sparse set of initial correspondences on the animal skin by selecting the skin points closest to a set of anatomical landmarks on the skeleton (e.g., the joints). From these sparse skin points, a dense set of correspondences is calculated by an iterative matching of local distributions of geodesic distances. This results in a set of point correspondences on the skin and on the skeleton that define the TPS interpolants. Figure 2 gives examples of this registration procedure in two mice.
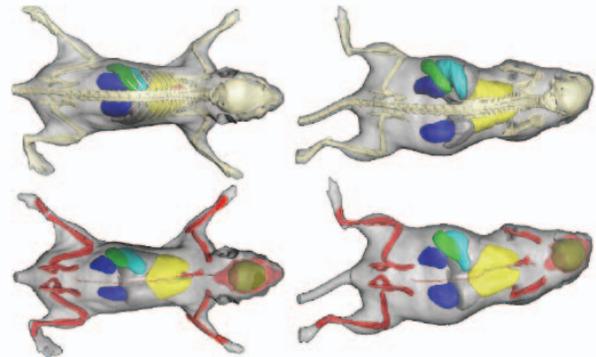
Figure 2 Manual segmentation (top row) versus automated skeleton registration and organ approximation (bottom row) for two mice in prone (left column) and supine position (right column).

This fully automated registration and organ approximation was validated in 41 μCT data sets [12]. A semi-automated generalization of this method towards μMRI data was described in [10]. Suh et al. developed a similar articulated registration approach for automated quantification of μSPECT –μCT data [11]

4. CHANGE DETECTION IN FOLLOW-UP STUDIES

Articulated atlas registration as described above can be applied to subsequent time points in a follow-up study. Similar to the curved planar reformation commonly used for visualization for tubular structures [17], we have introduced the Articulated Planar Reformation (APR) [18], where the raw CT data is reformatted along individual skeletal elements, and displayed in the atlas reference view. In this view, each subvolume corresponds to a single bone, which can be interactively selected for direct comparison with the corresponding sub-volume at the other timepoints. Additional visualizations are provided that automatically highlight areas of structural changes in the atlas view, based on the changes of bone density over time. Figure 3 demonstrates this approach on bone metastasis formation over time.

5. FUSION OF HETEROGENOUS IMAGE DATA

The articulated planar reformation can also be applied to multi-modal comparison of 3D datasets (Figure 4). However, for fusion of multi-view optical image data to structural imaging, 2D-3D registration is required based on matching the animal silhouette in the photographs to the 3D skin surface. In [19], we present a method for the registration of μCT to mouse skin surface to two or more 2D, geometrically calibrated, photographs of the same animal using a 3D distance map, which is reconstructed from the animal skin silhouettes in the 2D photographs. A robust registration criterion is derived by penalizing large angle differences between distance map gradients and CT-based skin surface normals that is insensitive to silhouette outliers and yields accurate results. Figure 5 gives an example of multi-view BLI to μCT registration; in addition the articulated atlas is fitted to the μCT. This way, an integrated data exploration is achieved, where the light-source outer envelope is coarsely approximated from the optical images for superficial lesions [20]. Anatomical context is provided by the fitted atlas. Figure 5 also visualizes the tumor location as seen in BLI in combination with bone destruction as seen in μCT data. Related work on registration of multi-view 2D photographs to 3D volumes is described in [21,22].

6. CONCLUSIONS AND FUTURE WORK

The presented approaches for whole-body registration, change detection and multi-modal fusion represent a first step towards 3D integrated analysis of whole-body small

Figure 3 A fully automatically derived articulated planar reformation visualization of timepoints at 3, 5 and 6 weeks, showing progressive bone destruction caused by metastatic bone disease (arrows). The femur is shown side-by-side in the focus views, though the animal posture in the raw data is highly variable.
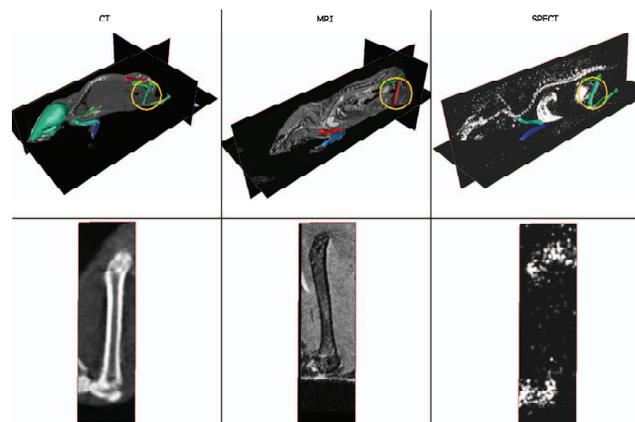
Figure 4. Articulated planar reformatting applied to μCT (left), μMRI (middle) and μSPECT (right) data from different mice.

animal follow-up studies. Validation studies reported elsewhere demonstrate high robustness of the developed registration methods [10,12,18,19], and end-users have reported benefits of the developed system in translational cancer research [3,4]. However, there are still many improvements that can be made towards minimizing the necessary user interaction, especially in multi-modal articulated registration. Also, a logical next step would be to integrate the articulated atlas registration into quantification methods for light sources in BLI and fluorescence imaging [23-25]. In general, such methods for photon attenuation and scatter correction greatly benefit from a subject specific tissue atlas.
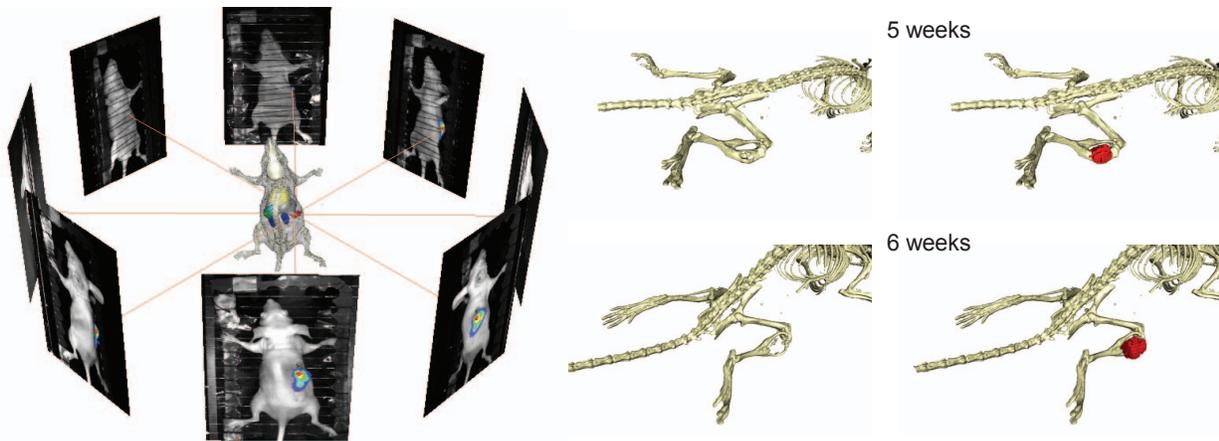
Figure 5: Left Panel: combined visualization of registered multi-view BLI data, μCT data and articulated atlas. Data can be interactively explored by pointing at lesions in either data type, where anatomical context is provided by the fitted atlas. Right panel: tumor location (in red) as visible with BLI, and associated bone destruction in μCT caused by osteolytic tumor activity.

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