Multi-Scale, Multi-Modal Fusion of Histological and MRI Volumes for Characterization of Lung Inflammation

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ABSTRACT

Mouse lung models facilitate the investigation of conditions such as chronic inflammation which are associated with common lung diseases. The multi-scale manifestation of lung inflammation prompted us to use multi-scale imaging - both \textit{in vivo}, \textit{ex vivo} MRI along with \textit{ex vivo} histology, for its study in a new quantitative way. Some imaging modalities, such as MRI, are non-invasive and capture macroscopic features of the pathology, while others, e.g. \textit{ex vivo} histology, depict detailed structures. Registering such multi-modal data to the same spatial coordinates will allow the construction of a comprehensive 3D model to enable the multi-scale study of diseases. Moreover, it may facilitate the identification and definition of quantitative of \textit{in vivo} imaging signatures for diseases and pathologic processes. We introduce a quantitative, image analytic framework to integrate \textit{in vivo} MR images of the entire mouse with \textit{ex vivo} histology of the lung alone, using lung \textit{ex vivo} MRI as conduit to facilitate their co-registration. In our framework, we first align the MR images by registering the \textit{in vivo} and \textit{ex vivo} MRI of the lung using an interactive rigid registration approach. Then we reconstruct the 3D volume of the \textit{ex vivo} histological specimen by efficient groupwise registration of the 2D slices. The resulting 3D histologic volume is subsequently registered to the MRI volumes by interactive rigid registration, directly to the \textit{ex vivo} MRI, and implicitly to \textit{in vivo} MRI. Qualitative evaluation of the registration framework was performed by comparing airway tree structures in \textit{ex vivo} MRI and \textit{ex vivo} histology where airways are visible and may be annotated. We present a use case for evaluation of our co-registration framework in the context of studying chronic inflammation in a diseased mouse.

Keywords: Mouse lung model, \textit{in vivo} MRI, \textit{ex vivo} MRI, branchi, histopathology, inflammation, chronic

1. INTRODUCTION

Often multi-modal images including MRI and CT allow for characterizing the lung pathology across different scales. However, multi-modal \textit{in vivo} imaging needs to be closely and tightly integrated with histopathology in order to be able to define and characterize imaging signatures of inflammation and other lung diseases. Additionally it is desirable to be able to merge the \textit{in vivo} imaging and \textit{ex vivo} pathology into an integrated canonical framework which would provide a unified, integrated multi-scale model for disease characterization. Such an integrated model would be valuable to characterize \textit{in vivo} imaging signatures of diseases.\textsuperscript{1-3} Recently, there has been great interest in developing fused multi-modal multi-scale models by combining \textit{ex vivo} and \textit{in vivo} imaging and pathology data to study diseases, such as prostate cancer\textsuperscript{1,4-6} and the brain.\textsuperscript{7,8}

Mouse models facilitate the study of various pulmonary diseases, e.g. chronic inflammation.\textsuperscript{9} Lung inflammation is a condition that can be visualized at different imaging scales from the thickening of the airways walls through the accumulations of foamy appearing alveolar macrophages.\textsuperscript{10} Methods to investigate \textit{in vivo} lung inflammation currently used include micro-CT\textsuperscript{11} as well as transillumination and fluorescence molecular tomography\textsuperscript{12} (see\textsuperscript{13} for a review). However, none of these studies involved a precise co-registration and fusion with \textit{ex vivo} histopathology. With pre-clinical models (e.g. mouse) there is the opportunity to quantitatively and rigorously evaluate imaging features of pathology by correlating the \textit{in vivo} imaging attributes with \textit{ex vivo} histopathology on a voxel-by-voxel basis. For example, \textit{in vivo} MRI shows macroscopic characteristics in three dimensions (3D), while histology captures higher level of details in two dimensions (2D) allowing the visualization of bronchi and their divisions within the lung.

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Multi-modal registration is however encumbered by a few significant challenges. Also, *ex vivo* histology images often suffer from artifacts caused by fixation and sectioning. Additionally *in vivo* imaging might suffer from deformation induced by the presence of other organs such as the heart. Consequently the multi-modal image registration algorithms need to be able to deal with the various sources of deformation induced within the different modalities.

While registration of *in vivo* imaging and *ex vivo* pathology images have been previously presented in the literature, the different approaches employed are typically a function of the type of pathology data available. For instance if only a few pathology sections are available, the only option to co-register with the *in vivo* imaging might be to first determine slice correspondences between the two modalities. This can then be followed by 2D-2D co-registration methods.\(^4,5\) Alternately if multiple concurrent histologic sections are available there might be an opportunity to reconstruct histologic volumes\(^14-17\) which could then allow for a true volumetric co-registration with the *in vivo* imaging.\(^6\) Such 3D-3D registration has the advantage of introducing additional spatial constrains to facilitate the accurate alignment. Also 3D-3D registration approaches for MRI and histology typically do not require establishment of slice correspondences from an expert.

In this work we introduce a co-registration framework that allows the integration of *in vivo* MRI and *ex vivo* histology data for the investigation of chronic inflammation in the lung. Our framework involves the co-registration of *in vivo* MRI, which shows the lung *in situ*, with *ex vivo* digitized histopathology of the entire lung. The 2D histological slices are collected from the excised lung at very fine intervals and are stained with hematoxylin and eosin (H&E) to capture inflammation at high resolution. Such high level of detail is needed, particularly in the case of inflammation, to visualize and characterize the pathology to be studied at a very fine, granular length scale. The inflammation may be manually annotated by a pathologist on each individual slice and serves as ground truth that is then mapped via our co-registration and image analytic framework on the corresponding *in vivo* MRI. Preceding the co-registration is a three dimensional (3D) reconstruction performed on the H&E slices to create the corresponding volume of the histology specimen.\(^17\) In addition to the *in vivo* MRI and histology slices, *ex vivo* MRI was also collected to visualize smaller structure, particularly airways, that may not be discernible *in vivo*; hence, the *ex vivo* MRI is used by the framework as a conduit to facilitate the accurate co-registration of *in vivo* MRI and corresponding *ex vivo* histology specimens. Our fusion framework allows us to construct a 3D imaging multiscale model of the lung, allowing us to quantitatively characterize inflammation on the *in vivo* imaging.

Our work introduces several novel contributions. (1) We present a fusion framework to reconstruct multi-scale 3D model of the mouse lung by integrating *ex vivo* histology with *in vivo* MRI. The solution is robust and allows to potentially include additional imaging modalities, such as micro CT or PET. (2) The multi-scale, multi-length scale model allows for quantitative identification of *in vivo* imaging signatures of chronic inflammation in a pre-clinical setting for the first time.

The remainder of the paper is organized as follows. The experimental design section describes first the data collected in the current study followed by the detailed description of the framework including the 3D reconstruction. Next, the results of the fusion are presented for each step in the framework. Finally, we discuss our results and present future directions.

## 2. EXPERIMENTAL DESIGN

### 2.1 Data Acquisition

In this work, two mice were considered: a mouse that lacks the surfactant protein D (SP-D) showing lung inflammation, and the wild type mouse without inflammation. Utilizing the Aspect MRI, the *in vivo* MRI data was collected on the peak inspiratory volume, which allows for the reconstruction of the *in situ* lung volume. Upon completion of the MRI, the mouse lung was extracted and inflated fixed with 4% paraformaldehyde. The fixed lung, while still in suspension within fluid, was examined by MRI using a 6-hour data collection window, which allows for a much higher resolution image to be gathered. The fixed lung was then embedded in paraffin and 5 \(\mu m\) sections were cut with a spacing of 55 \(\mu m\) (Figure 3). These sections were stained with Hematoxylin and Eosin (H&E) and examined using the Olympus VS120-SL scanning microscopy system.

Our mouse model is constructed using various images.
1. **Whole body in vivo MRI** has the lowest resolution and shows deformation of the lung due to the presence of other organs, e.g. heart or liver.

2. **Ex vivo MRI** shows the ex vivo lung at medium resolution allowing the visualization of the trachea and large bronchi, yet might show artifacts due to the sample preparation.

3. **Ex vivo histopathology slices** of the entire lung are imaged at high resolution; they may suffer from imaging artifacts due to sample preparation, e.g. folding and shrinking.

### 2.2 Annotation of pathology for ground truth extent determination

The ground truth extent for inflammation is determined via manual annotation on the ex vivo digitized histologic image sections. Inflammation is visible not only in the airways that are expected to thicken, but also at the level of the alveoli. Accurate delineation of inflammation is needed, such that a 3D map of inflammation can be identified. Accurate registration of the histology slices is thus needed to ensure a meaningful 3D map of the inflammation onto in vivo MRI.

### 2.3 Annotation of airways for qualitative validation

Along with the extent of inflammation, large airways are also delineated on each slice using a semi-automatic thresholding based approach. The segmentation of the large airways provides us the opportunity to (a) qualitatively evaluate the reconstruction results as well as to (b) guide the interactive registration of the histologic volume with the ex vivo MRI. Specifically, we used the airways to assess the tissue shrinkage caused by the slide preparation and staining.
Figure 2. One-to-one vs One-to-many registration. Each triangle symbolizes an H&E slice, while the number represents its order in the stack. Black arrow represents the registration direction, i.e. arrow heading on the template image. The difference between the one-to-one and one-to-many registration approaches is in the number of templates employed. Only one template is required in (a) allowing for fast piecewise image registration but may cause registration errors to propagate. On the other hand, groupwise registration in (b) uses multiple templates, requiring additional computations yet providing better global accuracy.

2.4 Registration of ex vivo MRI to in vivo MRI
First, the lung was manually segmented in both ex vivo MRI and the in vivo MRI. Then, the lung segmented in ex vivo MRI was rigidly aligned to the lung segmented in in vivo MRI using 3D Slicer. In our framework, the in vivo MRI is the reference fix image to which all other imaging protocols are aligned.

2.5 Reconstruction of the 3D histological volume
Several steps are employed in order to reconstruct the histological volume from the 2D histological slices. First, we apply one-to-one piecewise registration to generate an initial alignment of the slices which will be further optimized by the one-to-many groupwise approach. Given a set of 2D histological slices, adjacent slices are iteratively registered until all slices are aligned (Figure 2(a)). The alignment of two slices is achieved by maximizing the mutual information using rigid transformations. This piecewise step is able to quickly align the large number of slices corresponding to the lung. However with piecewise approaches, registration errors may propagate between slices resulting in slice drifting.

The groupwise step eliminates the drifting by considering more than one section as a template. Here we apply one-to-many groupwise registration. Given the set of slices aligned via the piecewise approach, we choose one slice that is most similar to the mean shape as an initial template and regard all others as moving slices to be registered (Figure 2(b)). A moving slice is first selected and aligned to the template such that the mean distance between the template and moving image is reduced (Algorithm 1). Next, the transformed moving slice is added to the template stack. Then, the next moving slice is selected and aligned to all the template slices. The process iterates until all moving slices have been aligned. The metric optimized here is the sum of squared differences between slices. We adopt the efficient Gauss-Newton optimization approach to minimize the metric. Since we align one moving slice to all previously aligned slices, our approach keeps the global consistency between slices during the registration.

2.6 Registration of the Histology Volume with ex vivo MRI
The 3D histology lung was aligned with the ex vivo MRI via interactive rigid registration using the outlined airways of the two modalities as landmarks to guide the alignment. Through the registration of the 3D histology lung and ex vivo MRI, it became apparent that the histological specimen has suffered considerable shrinkage during sample preparation. We assessed the amount of shrinkage by iteratively updating the scaling factor that was applied to the histological volume. For each scaling factor, the alignment of the airway tree structure in the ex vivo histology volume and ex vivo MRI was used to qualitatively estimate of the registration. In this work, we found a scaling factor of 2 to allow the best alignment between the histology volume and ex vivo MRI.
Data: a set of $N$ unaligned slices;
Result: aligned images;
Init: Pick the first slice as template;
for each unaligned slices do
    Pick one as the moving image while not converged do
        compute a small transformation displacement via gradient decent;
        add the displacement to the computed transformation;
        warp the moving image with the computed transformation;
    end
    foreach unaligned slices do
        warp the slice with the computed transformation;
    end
    add the aligned moving image as a new template;
end

Algorithm 1: Summary of our groupwise registration approach

the future, we consider acquiring additional data such as block face image of the histological sections before the sample preparation to accurately assess the scaling factor.6

Following these steps, the ex vivo histology specimen is aligned to the in vivo MRI. Such alignment enables the mapping of lung inflammation (annotated on histology slices) on to the in vivo MRI.

3. RESULTS AND DISCUSSION

Qualitative results are shown for each step of the framework (Figures 3-6).

3.1 Histology Volume Reconstruction

The groupwise registration allowed the building of the 3D volume representing the lung from the histopathology sections (Figure 3(b)). A careful inspection of the reconstruction reveals that the approach was able to align the slices without causing significant drifting between slices. Such drifting is visible when misalignment errors are propagated between consecutive slices and the model progressively rotates. Moreover, a fairly smooth surface of the volume suggests that the groupwise registration was able to properly identify the rigid transformations that were required to align the slices. Occasional minimal misalignment may be observed at the edges of the reconstruction, yet they are caused by the folding of the underlying tissue during the histology preparation. We also evaluate the alignment of slices by visual inspection of the airways (colored red in Figure 3(c)). When the proper transformation was identified, the airways were closely aligned by reconstructing the tree like structure.

3.2 Registration

3.2.1 Histology - ex vivo MRI registration

Following 3D reconstruction, the histological volume was registered to the ex vivo MRI, using iterative scaling followed by interactive rigid registration. Figure 4 shows the results of the histology volume fusion with ex vivo MRI. Although the airways align fairly well between the two modalities (Figure 4(b)), it may be observed that the lung surfaces appear misaligned between the ex vivo MRI and the corresponding 3D histologic reconstruction (green and purple surfaces in Figure 4(a)). Such misalignment is in part due to the imaging artifacts induced during the histology preparation, e.g. tissues shrinkage, and in the ex vivo MRI acquisition, e.g. large displacement of the lobes due to the fixation protocol.
3.2.2 In vivo and ex vivo MRI registration

The segmented lungs on the in vivo and ex vivo MRI were aligned using interactive rigid registration in 3D Slicer. Anatomic landmarks such as the trachea were utilized to guide the alignment. Overall, the shape of the lung was preserved between the two acquisitions (yellow versus green surfaces in Figure 5), allowing for a good alignment between the different lung surfaces.

Following the registration of ex vivo to in vivo MRI and subsequently co-registering the ex vivo MRI and the ex vivo histology, all 3 modalities were co-registered to a common reference frame allowing for the construction of a 3D imaging model of the lung in mice (Figure 6).
**CONCLUDING REMARKS**

In this work, we present a unified imaging framework for building a 3D imaging model of the lung with the goal of identifying imaging signatures of lung inflammation in vivo. The new framework was utilized to co-register the in vivo MRI with histology using the ex vivo MRI as conduit to facilitate the data fusion. The framework was applied here to create a 3D imaging model of a SP-D knock out mouse which shows lung inflammation, thus allowing for the mapping of regions of inflammation onto the corresponding in vivo MRI. Registration methods such as described in this work are required as lung inflammation shows a discontinuous distribution within the lung. In future work we intend to further develop our framework to allow incorporation of non-linear transformations and allowing for independent transformations for both sides of the lung. Moreover, we hope to increase the size of our cohort and utilize fully automatic registration approaches to integrate the multi-modal data. The co-registration framework will allow for direct measurement of changes that occur with inflammation, thus allowing for identification and evaluation of in vivo imaging signature of inflammation.

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