3D Histological Characterization of Precision-Cut Lung Slices for Inhalation Studies

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Abstract

Inhalation studies for allergens and pathogens typically rely upon flow cytometry which provides quantitative analysis of cell proteins associated with immune cells. However, flow cytometry does not provide information on the migration of these cells within the lung and is highly limited in describing the complexities of the lung. Due to these limitations, researchers have begun adopting precision cut lung slices (PCLS) as an *in vitro* tool as they are able to provide significantly improved *in vivo* relevancy. However, one of the current limitations in using PCLS models is that they are approx. 250 µm in thickness, and are too thick to image in their entirety using confocal microscopy. Therefore, it is challenging to capture data from these tissues past a few cell layers due to optical attenuation. Through this work, a rapid tissue clearing technique was applied to PCLS models in a high-throughput/well-plate-compatible format to enable whole mount 3D imaging of the entire PCLS model with confocal microscopy. It was shown that this tissue clearing approach was compatible with several commonly used labels (e.g. DAPI, CD68, tomato lectin, PDGFR, alpha-smooth muscle actin) and that uniform labeling as well as complete tissue characterization could be accomplished.

Introduction

The main rationale for the use and development of PCLS models is that the complex micro environment of the human lung is challenging to replicate in an *in vitro* setting, and such models have challenges with depicting the complex mechanical nature of the lung. Therefore, PCLS models allow for this replication in an *in vitro* setting with actual human-derived samples. However, these models are limited in practice as they require a donor from which the samples can be extracted and results in these samples being obtained on an ad hoc basis. Outside of sample procurement, the other challenge with PCLS models is their characterization as they are typically approx. 250 µm sections. This thick tissue section size means that traditional whole mount imaging depth with confocal microscopy is limited as light attenuates significantly through these samples. While these samples can be sectioned histologically, it is challenging to section a tissue so thin and fragile into sections from which histological information can be obtained. The other challenge with acquiring histological sections is that these models are expensive and difficult to obtain and thus it is ideal to obtain as much information as possible from these models when they used.

The Problem

The challenge with optical attenuation and PCLS models can clearly be seen below in **Figure 1** where a PCSL sample is mounted in PBS and then mounted in Visikol® HISTO-2™ which is a tissue clearing technique.

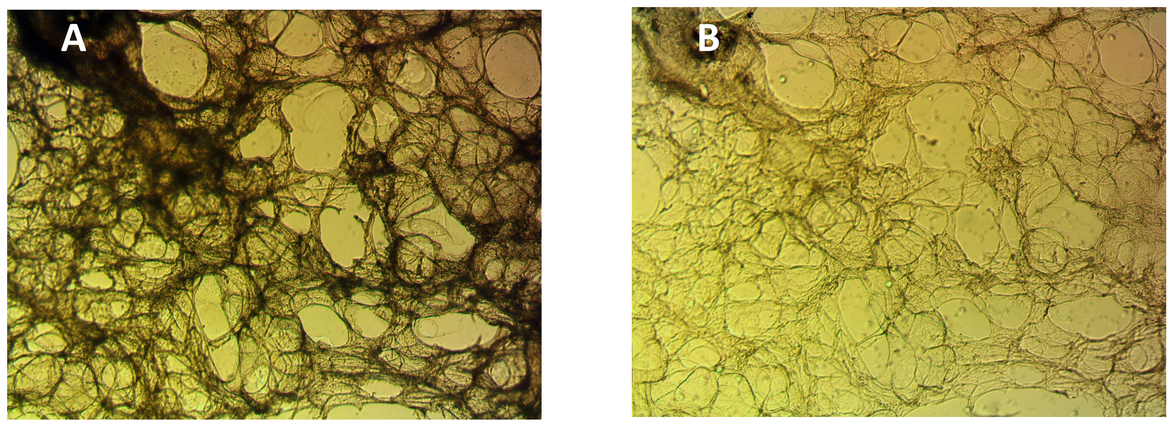


Figure 1. A) PCLS model that is mounted in PBS and imaged using wide field microscopy. B) PCLS model imaged in Figure 1A following tissue clearing with Visikol® HISTO™.

Materials and Methods

PCLS samples were acquired from a third-party vendor for use in this study. All tissues used in this study were imaged using a Nikon Eclipse 90i confocal microscope using green (488 nm), red (453 nm) and DAPI (405 nm) excitation. 2X, 10X and 20X magnification objectives were used for imaging and all objectives that were used were air objectives. Imaging was conducted using 512 and 2048 pixel width depending on the imaging requirements. Samples were labeled with CD68/Lectin or aSMA/PDGFR in accordance with the Visikol® HISTO™ protocol builder tool. For imaging, tissues were mounted in well-plates or imaged on glass microscope slides.

Results and Discussion

The major concern with conducting whole mount antibody labeling and whole mount labeling in general is that uniform labeling across the depth of the tissue will not be achieved. This is particularly problematic as non-uniform labeling will lead to an inability to extract meaningful data from tissues as the expression of labels will be indicative of non-uniform labeling and not the actual distribution of the marker for interest. Unlike many tissues, the lung is quite porous and uniform labeling across large distances such as within these PCLS models is achievable in a short period of time with little pre-treatment of tissues.

Through this work we demonstrated the ability to uniformly label across these tissues with small molecule chemical stains as well as large molecule antibody labels. This ability to label these samples in their entirety enables the three dimensional evaluation of these samples.

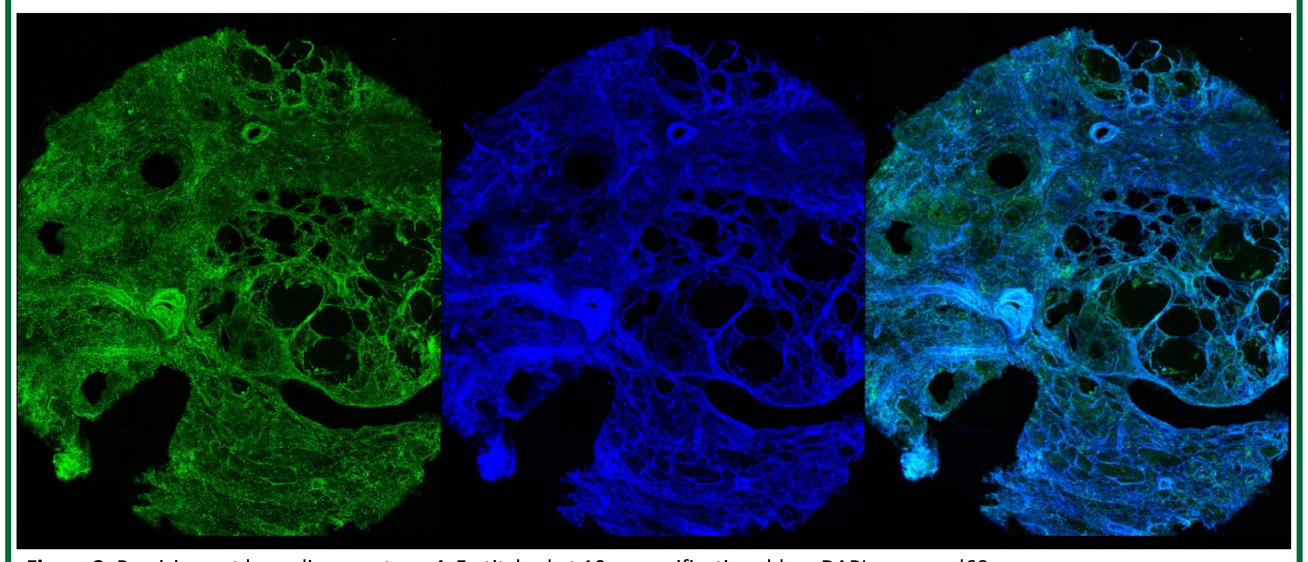


Figure 2. Precision cut lung slice montage-4x5-stitched at 10x magnification -blue=DAPI-green=cd68.

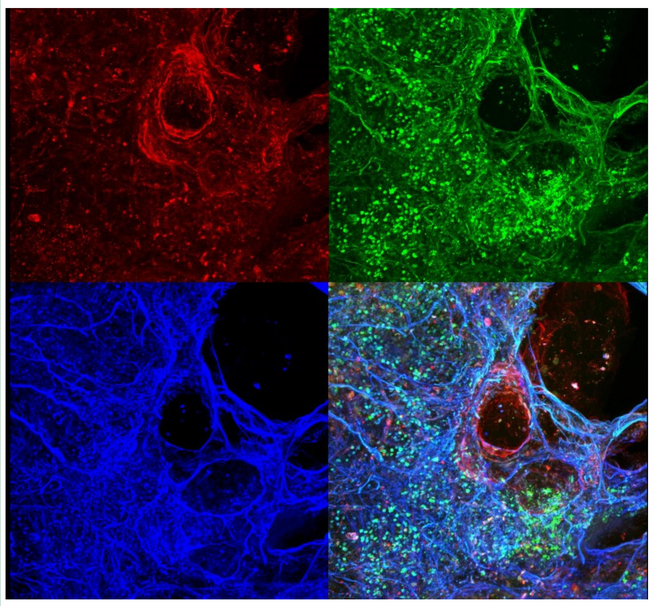


Figure 3. Precision cut lung slice montage z projection acquired at 20x magnification -blue=DAPI-green=cd68-red=lectin (RGB).

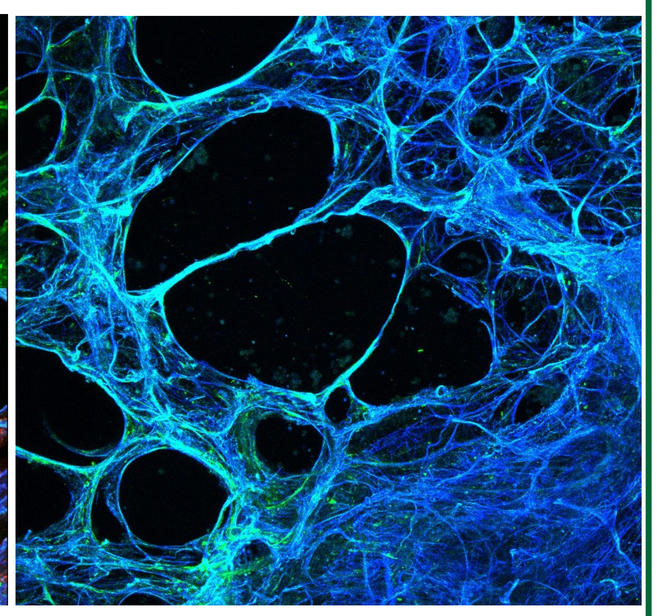
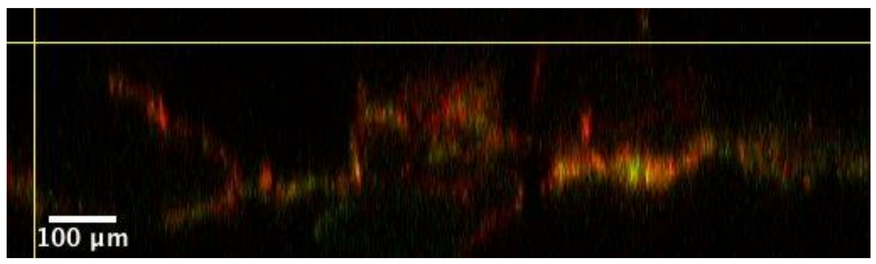


Figure 4. Precision cut lung slice z projection acquired at 10x magnification -blue=DAPI-green=aSMA-red=PDGFR.



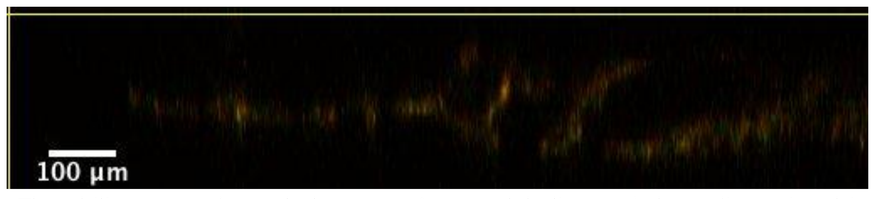


Figure 5. Precision cut lung slice quantification of imaging depth in a cleared and non cleared tissue sample. Sample that has been rendered transparent has a significant increase in imaging depth.

The ability to conduct whole mount immunolabeling and three-dimensional imaging of PCLS models using confocal microscopy allows for PCLS models to be profiled in their entirety and for these expensive in vitro models to be exhaustively utilized. The next practical step from this work is to leverage this methodology to develop a high-throughput PCLS processing and visualization workflow that can extract three-dimensional data from these models in an automated fashion. The Visikol® HISTO™ technology has been developed for automation and the staining and labeling of tissues can be entirely automated using liquid handling robots.

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