Whole Slide Digital Imaging with Image Quantitation of Lung Fibrosis in a Total Irradiation Mouse Model

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Digital whole slide imaging (DWSI) is a method to produce a digital virtual microscope slide. This digital image can be viewed in three or four fields, which are often used to evaluate the tissue. DWSI captures high quality digital images of histological slides and allows them to be stored in a database. As a result, researchers and clinicians can review the slides via their personal computer.

DWSI is useful in image analysis to determine the cell count, percent of tumor cell staining, and/or an object count of the field examined. This endeavor used the standard positive pixel algorithm, an FDA approved image analysis system for human clinical trials. The positive pixel algorithm is designed for immunostains and evaluates the intensity and area of brown staining in a slide. For a standard Masson’s Trichrome stained slide, the algorithm had to be altered and adapted to read the blue color as positive and red as negative rather than brown and blue respectively.

The goal of this study was to determine if the Aperio positive pixel algorithm could effectively quantitate mouse fibrosis in lung sections using Masson’s Trichrome stain. Slides from an irradiated mouse lung model were evaluated. Using fibrosis-prone C57BL/6 mice, lung slides were generated from control (non-irradiated) and irradiated mice subjected to 7-8 Gy dose of total body irradiation at 5 time points post-irradiation (1.4, 5.8, 6.4, 7.0, and 19.0 months), with at least 3 samples at each time point. The slides were scanned into the Aperio whole slide scanning system (Scanscope CS). The digital image was evaluated with the Aperio image analysis system using the standard positive pixel algorithm for the Trichrome image. These were compared against a standard pathology hand count of 0 to 8.

Whole slide Digital Imaging: The Aperio whole slide digital imaging system was used for imaging. The Aperio Scan Scope CS system was used (360 Park Center Drive Vista, CA 92081). All slides were imaged at 20x. Scan times ranged from 1 ½ minutes to a maximum time of 2.25 minutes. The whole images were housed and stored in Spectrum software system and images were shot from the whole slides.

Computer-assisted morphometric analysis of digital images was done using the included software of the Aperio Imaging system. An altered algorithm for the positive pixel algorithm that was set up for Trichrome staining was used for imaging of the Trichrome lung fibrosis. The positive pixel algorithm was modified to distinguish between the blue and red colors.

Automatic Image Quantitation: The Positive Pixel Count algorithm was used to quantify the amount of a specific stain present in a scanned slide image. The Positive Pixel Algorithm was altered with a hue value of 0.62, hue width of 0.40, and color saturation threshold of 0.005. A spectrum of color (range of hues and saturation) and intensity (weak, positive, and strong) were masked and evaluated.
Pathology Hand Count: The data were collected by counting the number of positive cells per high power field, then averaging the results of 3 fields per sample. The slides were given a ranking from 0-8. This was done in accordance with the guidelines set forth by Ashcroft, et al [1].

Using the Aperio positive pixel method, both whole slide and selected areas were analyzed for each Trichrome-stained slide (Figure 1). Average data for whole slide analysis indicated an increased level of fibrosis in irradiated mice compared with non-irradiated. Fibrosis also increased with age. The average positivity increased steadily after about 6.4 months post-irradiation in irradiated and after 7.0 months in non-irradiated controls. Selected area analysis was used to specify the area of analysis to the lung itself and to exclude areas that would result in false positives or false negatives. Although the data varies slightly in the selected area analysis, the overall trend is the same as in whole slide analysis, indicating that whole slide analysis is sufficient to determine the trend of lung fibrosis.

A standard pathology hand count was applied to the same Trichrome-stained slides as with the Aperio analysis (Figure 1). Using a 0-8 scale from Ashcroft, et al, there was little or no difference between irradiated and non-irradiated lungs at each time point. In addition, no increase in fibrosis was recognized until the 19 month time point. In contrast, the Aperio analysis showed a steady increase in lung fibrosis between roughly 6 months and 19 months in both irradiated and non-irradiated mice.

By comparing the Aperio positive pixel technique with the standard pathology hand count, it is clear that the Aperio analysis is a more sensitive method for evaluating fibrosis. In addition, because the Aperio analysis uses an unbiased algorithm instead of subjective criteria, the data are more representative of the gradual connective tissue deposition that occurs in a fibrotic lung. The numerical positivity scores generated using Aperio provide objective results that could be used in quantitative analyses and could easily be modified for other microscopy studies in the future.

![Graphs](image)

**Figure 1.** A comparison of methods for evaluating fibrosis in irradiated and non-irradiated mice was determined by whole slide Aperio analysis, selected area Aperio analysis, and with a standard pathology hand count.

References:
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