Novel morphometric image analysis of idiopathic pulmonary fibrosis models generates quantitative and continuous scores for the evaluation of fibrosis

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Introduction

Clinical drug development for treatment of Idiopathic Pulmonary Fibrosis (IPF) lack direct fibrosis endpoints. Lung histological evaluation using Ashcroft score captures fibrosis features and is only semi-quantitative. Therefore, new methods to measure fibrosis are under investigation such as using collagen fibers morphometry.

Objectives

To demonstrate the value of the FibroNest™ continuous Fibrosis Composite Score (FCS) to evaluate the severity of fibrosis in animal models and differentiate the Bleomycin from the Radiation. **Show that FCS has the potential to become a direct fibrotic endpoint.**

Methods

- Mice (n=2/grp) treated with Saline (Control)
- Mice (n=2/grp) with Bleomycin and Radiation induced lung fibrosis
- Second Harmonic Generation (Genesis200®) of 5 micron deparaffinized and unstained sections from both lungs
- Image analysis by FibroNest™ (V1.0, PharmaNest™, USA) for collagen content, structure and Morphometric phenotyping (40 quantified Fibrosis Parameters, qFP)
- Aggregation of the principal qFP in one or multiple composite scores
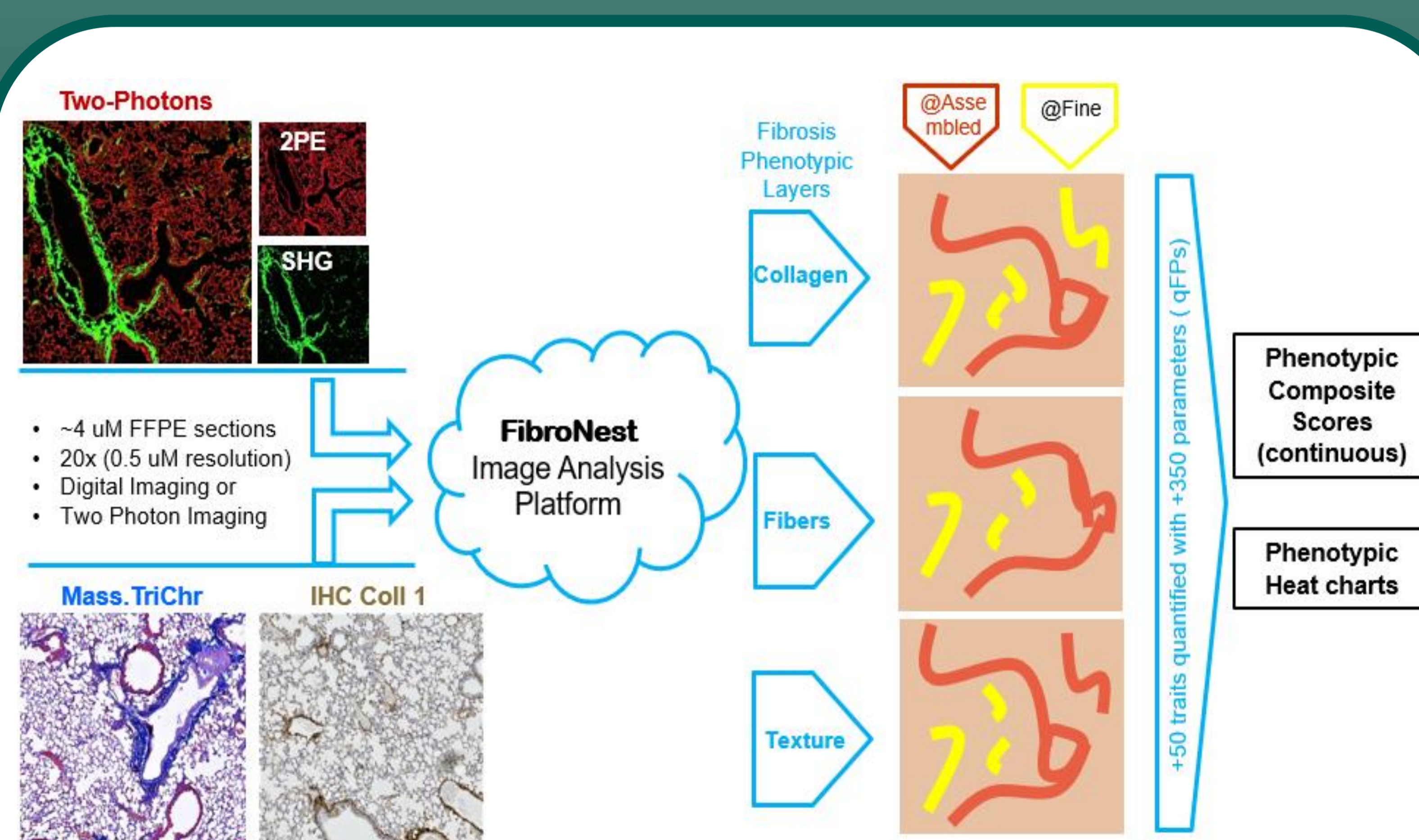- Heat Chart show the relative change of the principal qFPs

Figure 1: FibroNest™ workflow illustrated (image upload). FibroNest™ quantifies fibrosis at 3 levels, including collagen content, fibers morphometry and collagen texture.
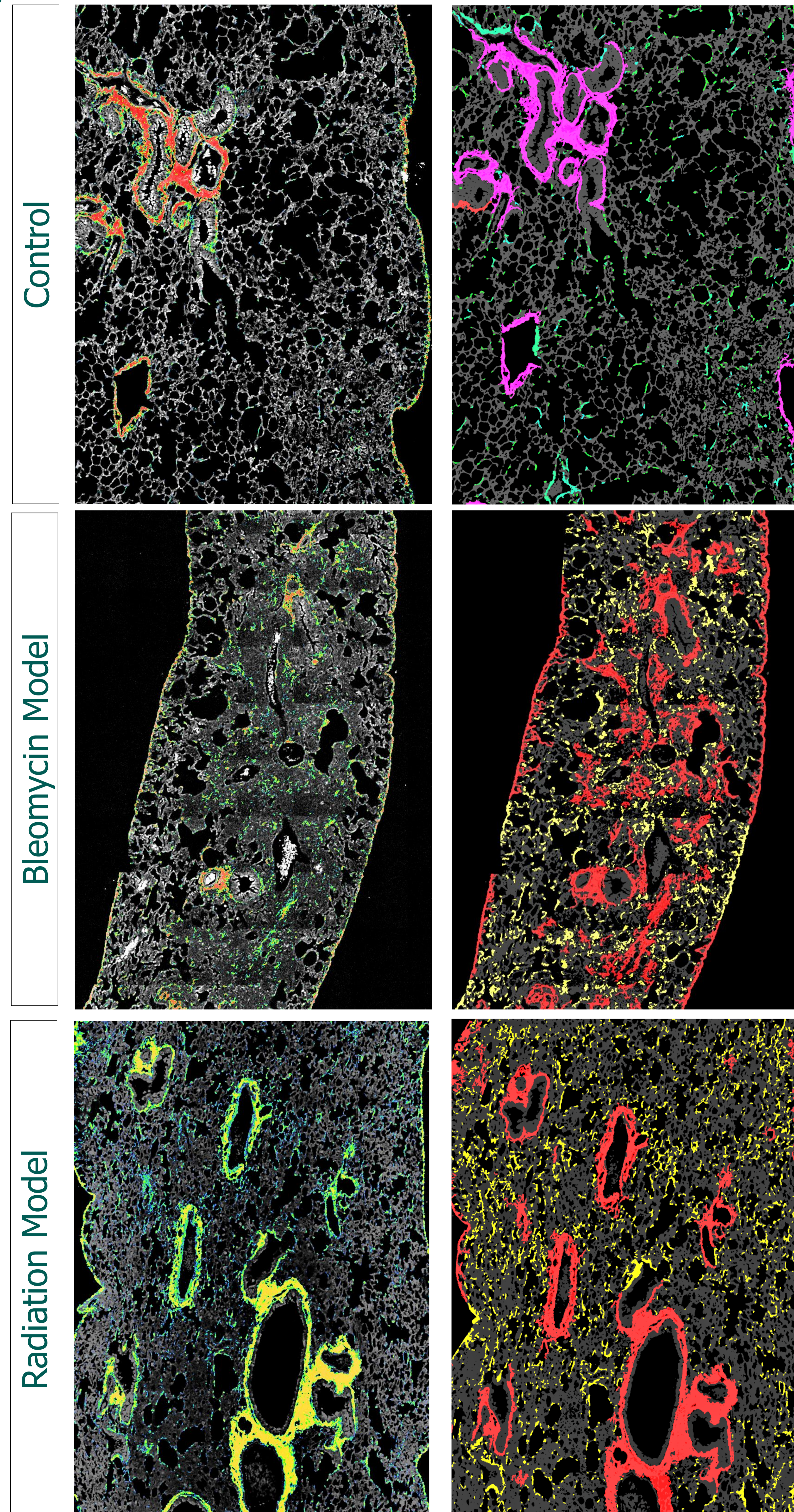
Results: Imaging and Analysis

Figure 2: <u>Left:</u> Two Photon images at 20x. White: 2PE tissue structure. and BioHeat color: Second Harmonic. <u>Right:</u> FibroNest™ image processing identify collagen features and classifies them into fine (yellow) and assembled (red) collagens

Results: Phenotypic Maps

Figure 3. Phenotypic Heatcharts (Green to Red severity scale for each Fibrosis quantifiable parameter). Each column are from the right and left lobes of the animal lung. Numerical values are combined into a Fibrosis Composite Score (FCS) shown on the last row.
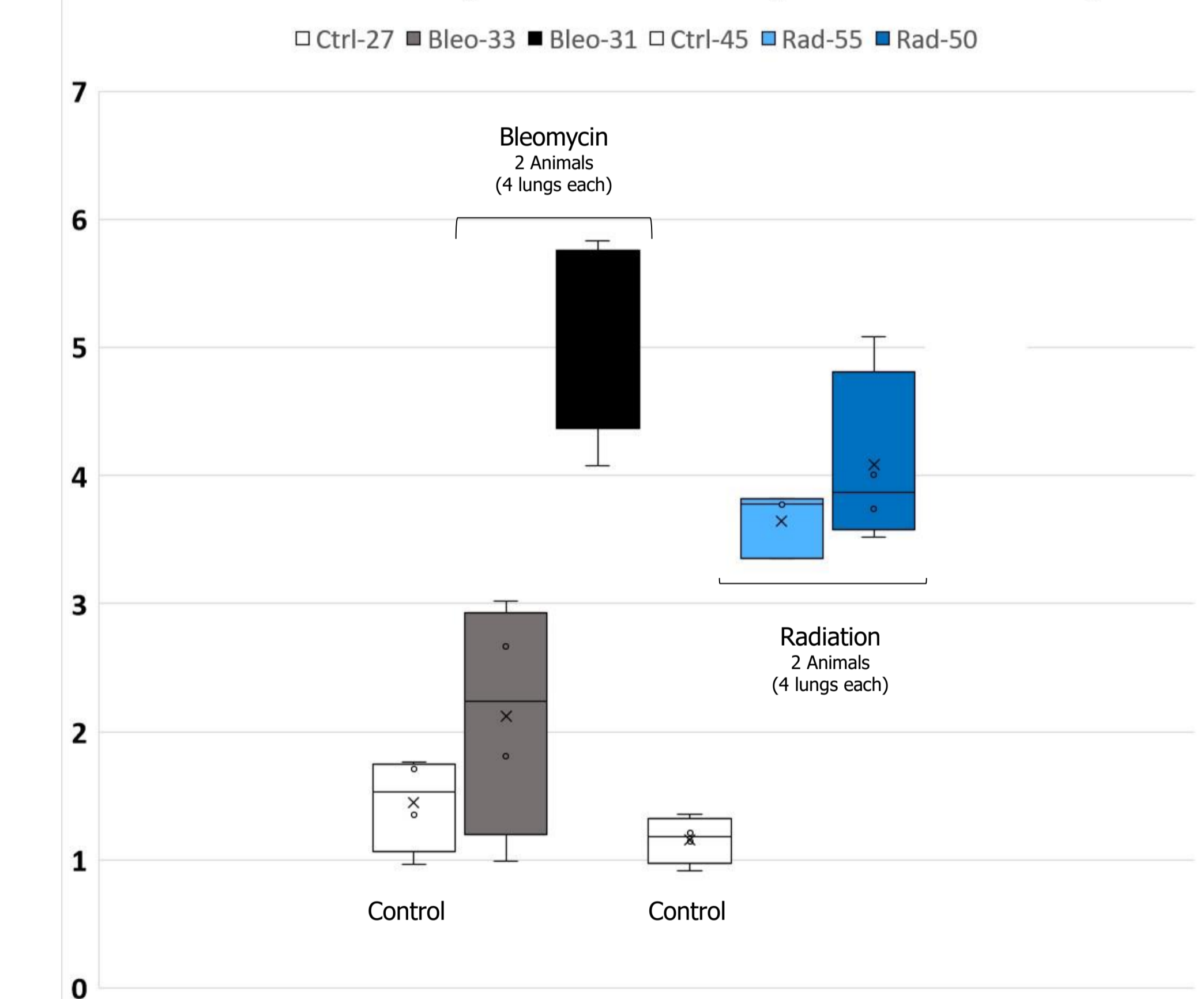

Figure 4. The Fibrosis Composite Scores (FCS) describe the variability of the severity of fibrosis across right and left lobes of the same animal, and the progression of fibrosis in each model. FCS account for the phenotypic differences between the Bleomycin and Radiation models. Subgroup analysis per specific lung show similar results.

Conclusions

The continuous Fibrosis Composite scores (FCS) established with FibroNest™ describe the severity of fibrosis for the Bleomycin and Radiation mouse models. If the FCS also show the response to a known anti-fibrotic compound, it has the potential to replace the existing categorical scores with poor intra / inter operator variability. FibroNest™ was able to quantify fibrosis phenotypes in two rodent models and can be compared to IPF biopsies that makes a method of choice to quantify and translate fibrosis endpoints in diseased lungs.

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