Second harmonic imaging (SHG) is a non-invasive technique used for studying biological samples for over a decade. Fibrillar collagen I, a very potent generator of SHG signal, is a component of the ECM and the most abundant mammalian protein. The contribution of extracellular matrix (ECM) remodelling to the pathogenic changes in chronic obstructive pulmonary disease (COPD) is complex and not well understood. Collagen I, a component of the ECM altered in COPD airways has second harmonic generation (SHG) properties. The SHG signal is coherent, propagating both forward (F) (primarily organized/mature collagen fibrils) and backward (B) (primarily disorganized/immature collagen fibrils) parallel to the incident light. Formalin fixed and paraffin embedded tissues of 30µm thickness was used in the experiments. F/B SHG ratio was used to determine the proportion of organized to disorganized collagen, with lower variation in F/B ratio between sampling regions within the same patient and between patients in the same disease group (Figure 1) compared to analysing F and B data alone. The F/B ratio was independent of laser power drift, regions analysed within a tissue and tissue orientation during analysis. Using this method we identified a significant difference in collagen organization in airway tissue between COPD and non-diseased patients. We have developed a robust optimization and calibration methodology that will allow direct comparison of data obtained at different times and from multiple microscopes that is directly adaptable for use with other tissue types. We report a powerful new tool for advancing our understanding of pathological ECM remodelling that may help uncover new therapeutic targets in the future.

References.

Acknowledgement: Cardiopulmonary transplant team and pathologists at St. Vincent’s Hospital. Surgeons and pathologists at RNSH, Concord and Strathfield Hospitals and Rhodes Pathology. J.K. Burgess is supported by a NHMRC Career Development Fellowship #1032695. Australian Microscopy and Microanalysis Facility
Fig. 1: Variation of Area F/B ratio values between regions (solid black lines, n=3 each patient), patients (distance between solid red lines, mean individual deviations from the averaged region variations from each patient for non-diseased or COPD) and disease (distance between blue dotted lines, relative difference of the means for non-diseased vs COPD).